The Molecular Basis of Lymphoid Architecture and B cell Responses: Implications for Immunodeficiency and Immunopathology

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Abstract: Immune responses usually take place in secondary lymphoid organs such as spleen and lymph nodes. Most lymphocytes within these organs are in transit, yet lymphoid organ structure is highly organized; T and B cells segregate into separate regions. B cell compartments include naïve cells within follicles, marginal zones and B-1 cells. Interactions between TNF family molecules on hematopoietic cells and their receptors on mesenchymal cells guide the initial phase of lymphoid organogenesis, and regulate chemokine secretion that mediates subsequent T-B cell segregation. Recruitment of B cells into different compartments depends on both the milieu established during organogenesis, and the threshold for B cell receptor signaling, which is modulated by numerous coreceptors.

Novel intrafollicular (germinal center) and extrafollicular (plasma cell) compartments are established when B cells respond to antigen. These divergent B cell responses are mediated by different patterns of gene expression, and influenced again by BCR signaling threshold and cellular interactions that depend on normal lymphoid architecture.

Aberrant B cell responses are reviewed in the light of these principles taking into account the molecular and architectural aspects of immunopathology. Histological features of immunodeficiency reflect defects of B cell recruitment or differentiation. B cell hyper-reactivity may arise from altered BCR signaling thresholds (autoimmunity), defects in stimuli that guide differentiation in response to antigen (follicular hyperplasia vs plasmacytosis), or defective B cell gene expression. Interestingly, in diseases such as rheumatoid arthritis, Sjögren's syndrome and Hashimoto's thyroiditis lymphoid organogenesis may be recapitulated in non-lymphoid parenchyma, under the influence of molecular interactions similar to those that operate during embryogenesis.

1. INTRODUCTION

1.1 Overview of Lymphoid Organs and B Cell Activation

B and T lymphocytes are distinguished from other cells of the immune system, and indeed all other somatic cells in the body, by their capacity to activate gene recombination. Genes encoding antigen receptors - immunoglobulin in B cell receptors, and T cell receptors - undergo recombination during lymphocyte development. Each lymphocyte expresses only one type of antigen receptor [1]. However, the stochastic recombination process results in tremendous diversity of receptors across the repertoire of lymphocytes (reviewed in [2]). There are two important trade-offs for antigen-receptor diversity. First, lymphocytes specific for a particular antigen are rare, and after activation these rare precursors must undergo clonal expansion if sufficient antigen-specific effector cells are to be generated. Consequently, there is a delay between lymphocyte activation (antigen recognition) and the response that clears the pathogen. Second, a stochastic process of receptor diversification carries a risk of generating autoreactive lymphocytes, and if these remain in the repertoire and become activated they can cause autoimmune disease.

In contrast to T and B cells, the innate immune system comprises cells and molecules that use antigen receptors encoded by germ-line genes and receptor diversity is limited. Components of innate defense include phagocytes, natural killer cells, complement, C-reactive protein, and kinins. These receptors recognize molecular patterns common to pathogens but absent from host cells. Since the number of cells and molecules that recognize any particular foreign antigen is large, the innate system can provide rapid early defense. Consequently, for any particular pathogen, specific responses by T

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and B cells are usually established after innate immune responses.

B cells produce antibodies, which form a crucial defense mechanism against antigens within the extracellular space. Antibodies can eliminate pathogens by several mechanisms including complement activation and direct lysis of bacteria (eg meningococcus), neutralization of viruses and toxins to prevent their entry into host cells (eg enteroviruses and tetanus toxin), and opsonization (eg *E. coli*). These effector mechanisms illustrate that innate and specific responses do not operate independently; antibodies combine with components of the innate response such as complement and phagocytes. The specific immune response is also influenced by the innate response at the time of lymphocyte activation, and there exists an ongoing and complex interplay between the two systems throughout immune responses.

From this discussion, several points emerge about the nature of B cell responses. First, B cell activation depends on interactions between antigen-specific cells that are rare within a large repertoire. Second, successful immune defense relies on complex interactions between many different components of the immune system. Third, there is the danger that the response may go awry and result in immunopathology. These features provide insights into the selective advantage conferred by the evolution of sophisticated spatio-temporal organization of B cell responses.

Most immune responses take place in secondary lymphoid organs such as lymph nodes and spleen, where resting T and B lymphocytes are compartmentalized and activated lymphocytes are segregated as they proliferate and differentiate. Compartmentalization helps to explain how interactions between rare antigen-specific cells can occur, and also how components of the innate immune system influence specific immune responses without compromising the specificity of these responses. Segregation of activated B cells reflects the necessary specificity of cellular and molecular interactions for efficient differentiation into effector and memory cells.

1.2 Normal B Cell Migration

Secondary lymphoid organs include lymph nodes, Peyer's patches, tonsils and adenoids, mucosa-associated lymphoid tissue, and the white pulp of the spleen. Secondary lymphoid organs provide points of intersection between naïve and antigen-experienced lymphocytes, accessory cells and molecules from the innate immune system, and antigen. In contrast to other organs where cells are cemented together, lymphoid organ parenchyma comprises cellular constituents (mostly lymphocytes) that are only temporary residents. Naïve lymphocytes recirculate continuously between different lymphoid organs, Fig. (**1**). Within secondary lymphoid organs, naïve lymphocytes make transient interactions with each other and with the stromal components of the organs.

In the secondary lymphoid organs, T and B cells segregate into separate zones. B cells are organized into follicles, which are spheroid aggregates located in the cortex of lymph nodes, in the domes of Peyer's patches, and exterior to periarteriolar lymphoid sheaths of T cells in the white pulp of the spleen (reviewed in [3]). While B cells predominate, follicles also contain resident follicular dendritic cells (FDC) and a small number of T cells.

Continuous cellular migration facilitates interaction between different cellular components. For example, T cells peruse the surface of interdigitating dendritic cells for peptide/major histocompatibility complex (MHC) complexes with which to make cognate interactions [4]. Such interactions prime naïve T cells. When primed T cells ligate the same peptide/MHC complexes on the surfaces of B cells, they can provide B cells with 'helper signals' that are necessary for B cell activation [5]. B cell activation usually requires an interaction between B cells and T cells that recognize the same antigen. This unlikely interaction is plausible because antigen-stimulated B cells migrate to the interface between T and B cell zones.

Once activated, B cells proliferate and differentiate and the type of antigenic stimulus and the help available determines the nature of differentiation. One pathway leads to rapid antibody formation from short-lived plasma cells generated outside follicles. Another pathway leads to seeding of the follicles with blasts that undergo massive expansion, forming germinal centers *(Section 5.5)*. Proliferation within this environment is associated with somatic hypermutation of immunoglobulin variable region (IgV) genes and results in the production of high affinity memory and plasma cells [6]. This process is thought to involve interactions between B cells, T cells, and FDC.

Afferent lymphatics drain most tissues in the body with the exception of so-called sites of immune privilege such as eye, brain, and testis. Afferent lymphatics carry antigen and antigen-presenting cells to the draining lymph nodes. Superficial lymph nodes drain into deeper lymph nodes. Naive lymphocytes enter lymph nodes via the high endothelial (post-capillary) venules in the paracortex (T cell zone) [7]. Some memory and effector lymphocytes also enter lymph nodes from the bloodstream via the high endothelial venules, while others emigrating from non-lymphoid organs enter through the afferent lymphatics that open into the subcapsular (or marginal) sinus located

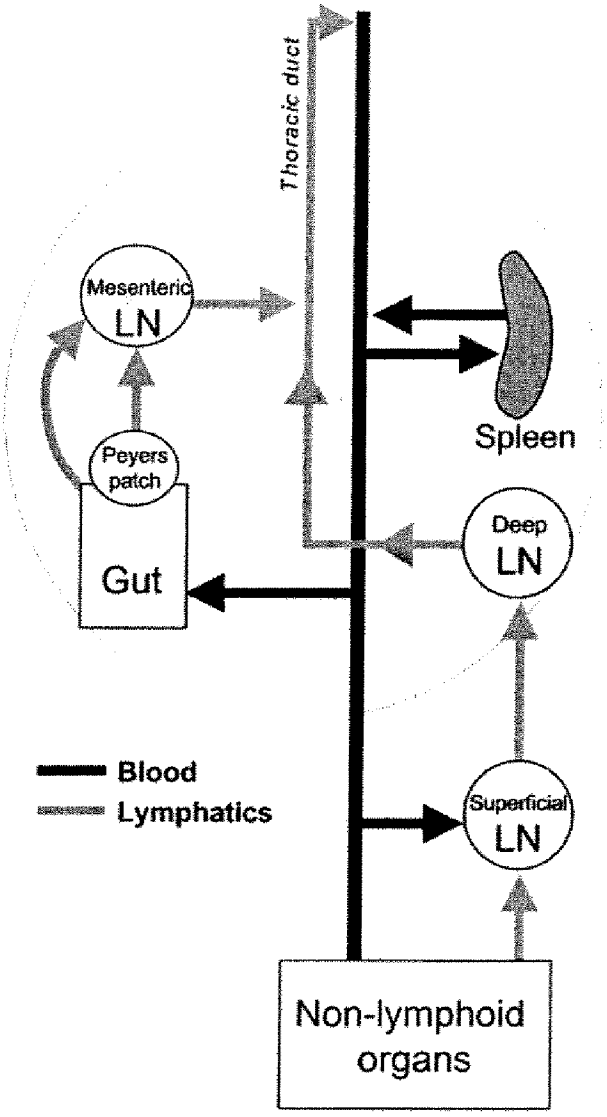

Figure (1). Summary of lymphocyte recirculation.

Lymphocytes circulate in the blood and enter lymph nodes and Peyer's patches via high endothelial venules. From peripheral nodes, lymph drains centrally via the efferent lymphatics and deeper lymph nodes, eventually reaching the thoracic duct and returning to the systemic circulation. Lymphatics from the gut mucosa drain via Peyer's patches and also directly into mesenteric lymph nodes. The spleen is not connected to the lymphatic system and lymphocytes enter and leave exclusively via the blood.

immediately beneath the capsule on the convex surface of the node. In addition, antigen-presenting cells migrating from non-lymphoid parenchyma enter lymph nodes via the afferent lymphatics, and free antigen also filters into the lymph nodes via the afferent lymphatics and may be trapped by macrophages and dendritic cells located on the fibrous septa of the subcapsular sinuses. The subcapsular sinus drains via intranodal sinuses towards the medulla, draining ultimately into the efferent lymphatics that arise from the node hilum, Fig. (**2a**). Antigen presenting cells travel from Peyer's patches and directly from gut mucosa via lymphatic vessels into mesenteric lymph nodes [8, 9].

The spleen is divided into two compartments with different functions. The red pulp comprises venous sinuses and contains macrophages that remove senescent red blood cells from the circulation. The white pulp is the lymphoid component of the spleen, comprising follicles, T cell zones (also known as periarteriolar lymphoid sheaths) and marginal zones. The marginal zone,

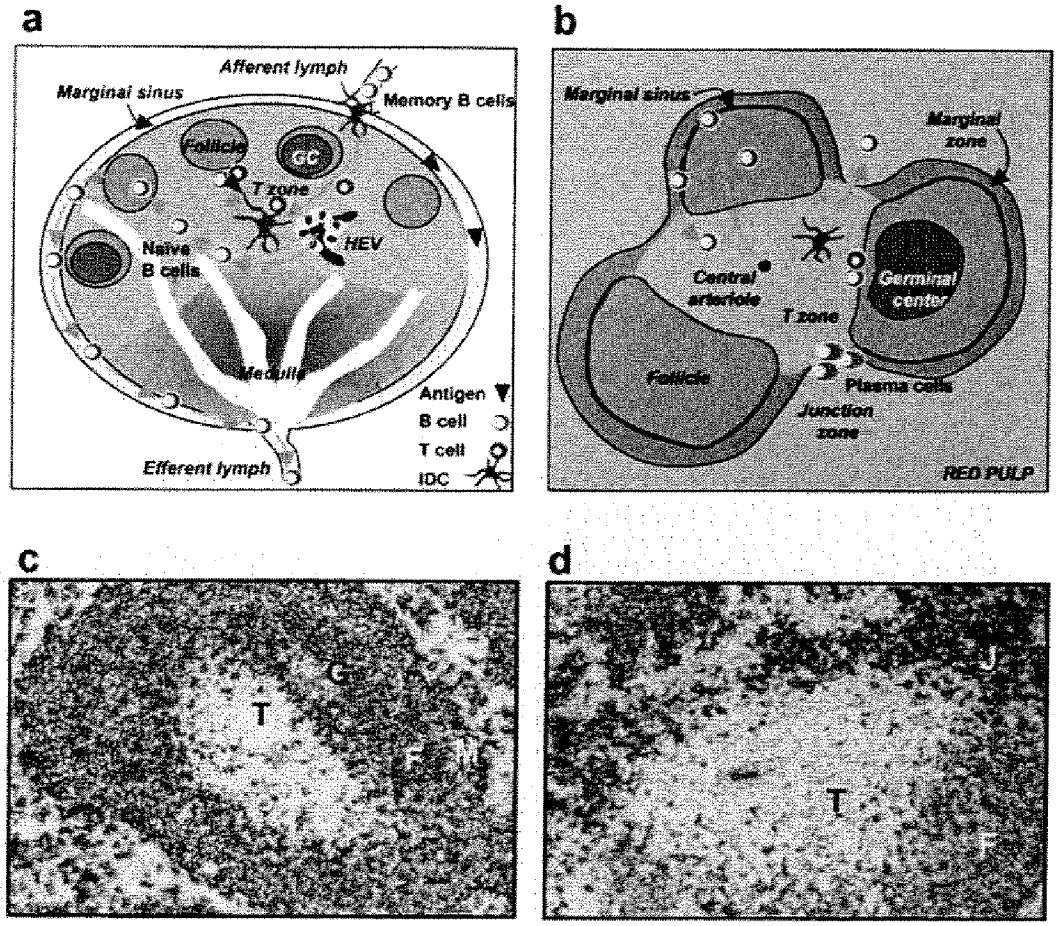

Figure (2). Architecture of secondary lymphoid organs.

(a). Lymph node: Afferent lymphatics deliver memory lymphocytes, antigen and dendritic cells to the marginal (subcapsular) sinus. Naïve B cells enter via high endothelial venules, which open into the paracortical region. Naïve B cells are organized into follicles in the cortex. T cells are segregated into paracortical regions. Antigen stimulated B cells migrate to the outer T zone (adjacent to the follicles). In the presence of T cell help they proliferate and differentiate. One pathway of differentiation gives rise to germinal center B cells within follicles, the other to plasma cells in the medullary cords (not shown). Unstimulated B cells migrate through follicles and eventually leave via the efferent lymphatics. (b). Spleen: Lymphocytes enter the while pulp via the central arteriole and penicillary branches that open into the marginal sinus located between the marginal zone and follicle. B cells drain via the T zone to the follicles. Contact with antigen causes B cells to arrest in the outer T zone. Antigen stimulated B cells differentiate into germinal centers in follicles and extrafollicular plasma cells in junction zones between red and white pulp. Unstimulated B cells drain via venous sinuses in the red pulp and return to the systemic circulation. (c). Spleen white pulp histology: Photomicrograph of section of mouse spleen stained for IgD (brown) and IgM (blue), to identify follicles (F) and marginal zone (M). Proliferating cells stain red (bromodeoxyuridine), and most of these are located within a germinal center (G). (d). Spleen white pulp histology: Photomicrograph of section of mouse spleen stained for IgD (brown), syndecan-1 (blue, plasma cells), bromodeoxyuridine (pink) to identify plasmablasts in the junction zones (J).

which is unique to the spleen, is located between the white pulp and the red pulp and contains specialized macrophages and B cells.

Since there are neither afferent lymphatics nor high endothelial venules in the spleen, antigen and lymphocytes can only enter via the arterial circulation. The white pulp is perfused by central arterioles located within the centers of the T zones. Central arterioles give off branches that open into the marginal sinuses, which are loose endothelial structures located between the white pulp and marginal zones [10, 11]. After entry in the marginal sinuses, B cells migrate via the T zones, through the

follicles and into the red pulp. From here they enter venous sinuses in the red pulp that drain to the venous circulation. Antigen also enters via the marginal sinus, where it comes into contact with macrophages and marginal zone B cells, before diffusing into the phagocytic system of the red pulp (cords of Billroth), Figs. (**2b, c, d**).

1.3 Importance of Secondary Lymphoid Organs for Immunity

Immune responses are perturbed or even abolished when specialized immune compartments are absent. The importance of afferent lymphatics for initiation of normal immune responses was suggested by early data [12]. These experiments can now be interpreted as demonstrating the role of migrating dendritic cells for initiation of T cell activation. Other experiments have demonstrated that selective absence of the spleen results in a defective antibody response to encapsulated organisms [13, 14] (*Section 6.3*). In the absence of secondary lymphoid organs, normal immune responses to some viruses are delayed significantly, and contact hypersensitivity responses to epidermal haptens are abrogated altogether [15, 16]. Furthermore, in mice lacking both spleen and lymph nodes, allografts are tolerated indefinitely [17].

2. MOLECULAR REGULATION OF SECONDARY LYMPHOID ORGAN DEVELOPMENT

2.1 Lymph Node Development

Colonization of lymph node anlagen with lymphoid cells is a two-stage process. First, hematopoietic progenitor lymphoid cells interact with endothelium and mesenchymal cells to colonize the primordial lymph node. The initial interaction appears to be mediated by binding of adhesion molecules on high endothelial venules in primitive lymph nodes to ligands on hematopoietic precursors. Second, binding of TNF family molecules on hematopoietic cells to their receptors on mesenchymal cells leads to local release of chemokines, which triggers a second phase of development in which mature lymphocytes are recruited to the nascent organ. Newly recruited lymphocytes also contribute by inducing maturation of mesenchymal elements within the lymph node, which is necessary for formation of B cell follicles that can support normal immune responses. These phases are discussed in detail below and summarized in Fig. (**3**).

2.2 Lymphoid Precursor Cells and Their Recruitment to Primordial Lymph Nodes

Organogenesis of peripheral and mesenteric lymph nodes, Peyer's patches, and possibly splenic white pulp as well, appears to be initiated by an unusual population of lymphoid progenitor cells, identified in fetal blood and liver as $CD45^+$ $CD4^+$ $CD3^-$ $\alpha_4\beta_7^+$ $LT\alpha_1\beta_2^+$ $CXCR5^+$ $IL\text{-}7R\alpha^+$ [18]. These lymphoid progenitor cells give rise to macrophages, dendritic cells, and NK cells, but not T and B cells. Once these cells enter the early lymphoid structure, it is likely that their crucial lymph node organising function is at least in part due to their expression of LTβ and CXCR5 (discussed below). More recently, a population of $IL\text{-}7R\alpha^+$ $Sca\text{-}1^{lo}$ $c\text{-}Kit^{lo}$ progenitor cells has been isolated from fetal liver, which can generate not only the lymphoid progenitor cells found in adult bone marrow, but T and B cells as well [19].

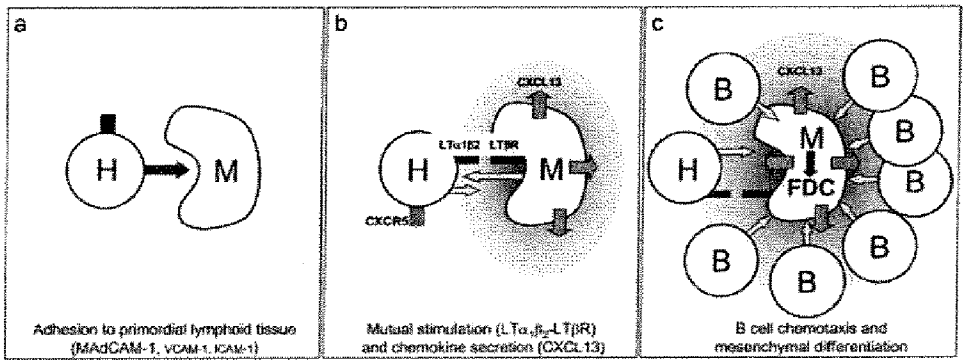

Figure (3). Summary of receptor ligand interactions of TNF family members important for lymphoid organogenesis.

See text for details.

Additional evidence that $CD45^+$ $CD4^+$ $CD3^-$ $\alpha_4\beta_7^+$ progenitor cells play a central role in lymphoid organogenesis has been obtained from separate strains of mutant mice that lack this population. Retinoid orphan receptor-γ ($ROR\gamma$)-deficient mice exhibit extensive thymic apoptosis and also lack lymph nodes and Peyer's patches [20]. $Id2^{-/-}$ mice lack lymph nodes and Peyer's patches but have normal splenic architecture [21]. Both mutant strains lack the $CD45^+$ $CD4^+$ $CD3^-$ IL-$7R\alpha^+$ lymphoid progenitors.

$CD45^+$ $CD4^+$ $CD3^-$ $\alpha_4\beta_7^+$ $LT\alpha_1\beta_2^+$ $CXCR5^+$ lymphoid progenitor cells are selectively recruited by interactions with adhesion molecules expressed by high endothelial venules in primordial lymphoid organs. Selection of rare progenitors from blood proceeds by virtue of the fetal program of adhesion molecule expression that mediates leukocyte homing to lymph nodes. As noted above, lymphocytes leave the blood stream to enter lymph nodes by crossing the specialized high endothelial venules, which express cellular adhesion molecules that ligate receptors on leukocytes. In adults, high endothelial venules of peripheral lymph nodes express peripheral lymph node addressin (PNAd) and MAdCAM-1 and recruitment of recirculating lymphocytes is mediated by L-selectin binding to PNAd [22]. Although $\alpha_4\beta_7$ is also a ligand for MAdCAM-1, it appears to be redundant for lymphocytes to home to peripheral lymph nodes in adults. By contrast, during embryogenesis the interaction between $\alpha_4\beta_7$ and MAdCAM-1 enables peripheral lymph nodes to be populated [23] with $\alpha_4\beta_7^+$ lymphoid progenitor cells. Early in the neonatal period there is a switch in the preference of peripheral lymph nodes from $\alpha_4\beta_7^+$ cells to L-$selectin^+$ cells, which enhances recruitment of mature lymphocytes.

Further evidence to support the requirement of cell adhesion events is provided by studies of the homeodomain factor NKX2-3, which is expressed in the mesoderm of gut and spleen during development. $NKX2\text{-}3^{-/-}$ mice exhibit several developmental defects, including reduced expression of MAdCAM-1 on HEV of Peyer's patches, marginal sinus endothelium, and germinal centers within the spleen [24]. Reduced embryonal MAdCAM-1 expression in spleen white pulp, Peyer's patches and mesenteric lymph nodes correlates with marked disruption of their normal architecture, whereas in peripheral lymph nodes, where MAdCAM-1 expression is unaffected, normal architecture is preserved.

$CD45^+$ $CD4^+$ $CD3^-$ $LT\alpha_1\beta_2^+$ IL-$7R\alpha^+$ precursor cell recruitment is also crucial for Peyer's patch development, and cellular interactions during Peyer's patch development may provide insights into lymph node development and ectopic lymphoid neogenesis (*Section 6.3.3*). In adults, lymphocyte recruitment to Peyer's patches is mediated by $\alpha_4\beta_7$ binding to MAdCAM-1 on high endothelial venules. By contrast, during embryogenesis $\alpha_4\beta_7^+$ progenitor cells bind to VCAM-1 and ICAM-1, which are expressed on mesenchymal elements in Peyer's patch anlagen. VCAM-1/ICAM-1 expression is IL-7Rα-dependent and mice deficient in IL-7Rα, or the downstream signaling molecule Jak3, lack Peyer's patches [25]. The source of IL-7 remains uncertain. Ligation of IL-7Rα on progenitor cells induces expression of $LT\alpha_1\beta_2$ and chemokines that promote lymphoid colonization of the developing Peyer's patches. This may reflect induction of CXCL13 expression within the mesenchymal elements, since progenitor cells express CXCR5 and both $CXCL13^{-/-}$ and $CXCR5^{-/-}$ mice lack most peripheral lymph nodes and Peyer's patches (see *Section 3.2*) [26-28]. VCAM-1 is also expressed on fetal lymph node high endothelial venules and ligation of this molecule may contribute to precursor cell recruitment to primordial lymph nodes, since MAdCAM-1 blockade fails to completely abrogate progenitor recruitment [23].

2.3 Ligation of LTβR on Mesenchymal Cells During Lymph Node Development

After recruitment of progenitor cells, recruitment of other populations and subsequent organization of cell populations within secondary lymphoid tissue depends on members of the TNF family. The roles of TNF, lymphotoxin (LT)-α and LTβ have been characterized most thoroughly but the list of ligands, receptors and downstream signaling molecules that mediate lymph node and spleen development continues to grow.

There are two TNF receptors: type I TNF receptor (TNFRI, p55) and type II TNF receptor (TNFRII, p75), Fig. (**4**). These are expressed ubiquitously on cell surfaces and also exist in circulating soluble forms that compete with transmembranous receptors for ligand binding and can inhibit cell-to-cell interactions. Expression of transmembranous TNF is limited mainly to macrophages, natural killer (NK) cells and activated T and B lymphocytes. LTα is expressed by activated T, B and NK cells. Transmembranous TNF and LTα both exist as homotrimers (TNF_3, $LT\alpha_3$) that bind TNFRI and TNFRII with similar affinity, although transmembranous TNF appears to preferentially ligate TNFRII [29]. TNF and LTα also exist as circulating (soluble) cytokines, generated when membrane-bound molecules are cleaved by a specific metalloproteinase (TACE) [30]. LTβ does not exist as a free or transmembrane ligand, but associates with LTα to form heterotrimers. $LT\alpha_2\beta_1$ binds to TNFRI or TNFRII, whereas $LT\alpha_1\beta_2$ shows no affinity for either TNF receptor, but binds to a distinct cellular receptor designated LTβR [31]. Significantly, lymphoid cells do not express LTβR.

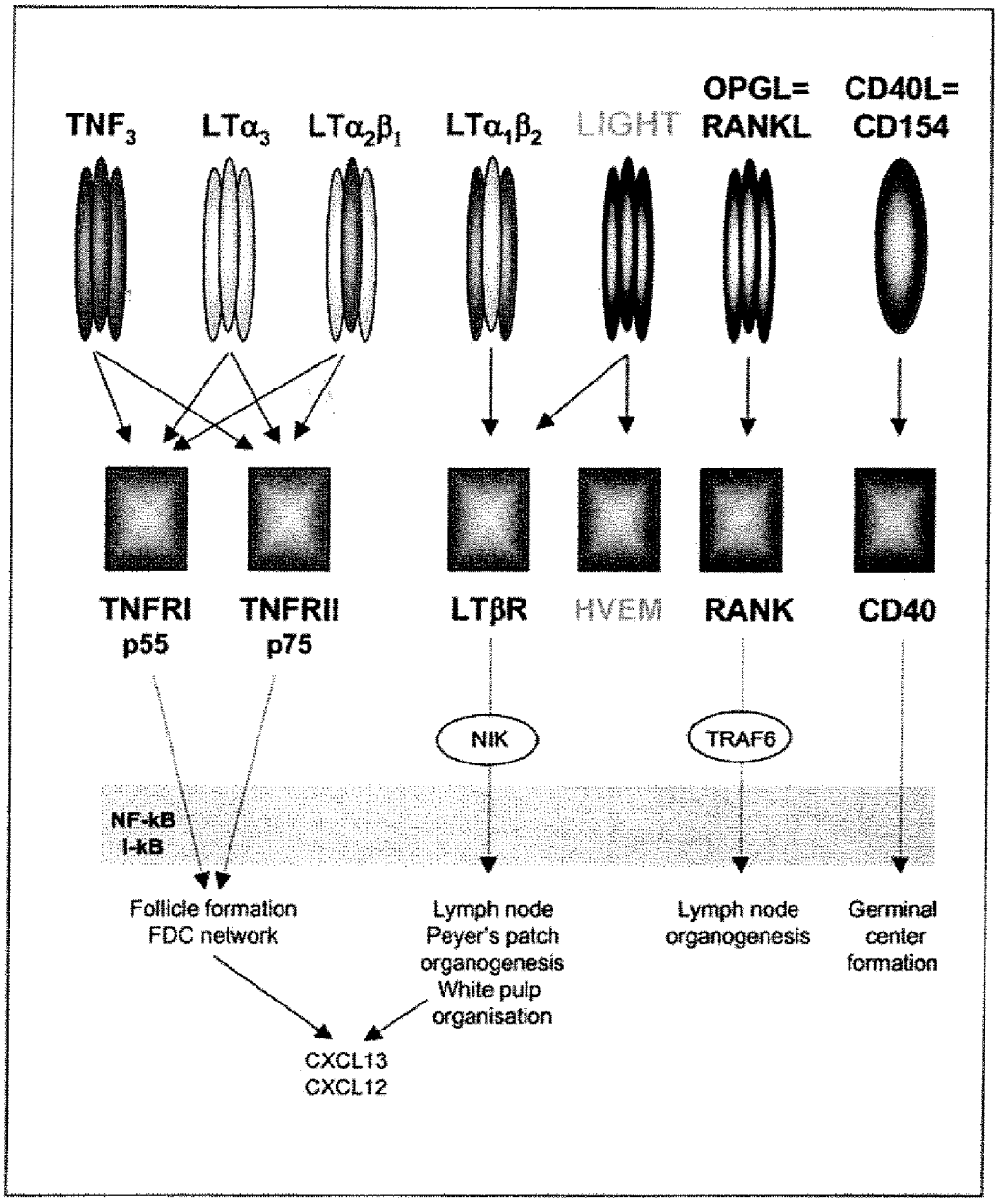

Figure (4). Overview of lymphoid organogenesis.

(a). Hematopoietic lymphoid progenitor cells bind to MAdCAM-1 on high endothelial venules in the primordial lymph node. (b). $LT\alpha_1\beta_2$ on the hematopoietic cells ligates LTβR on the mesenchymal cell, inducing chemokine secretion. (c). Chemokines attract mature B cells into the lymph node. B cells are organized into follicles under the influence of secreted chemokines. B cells induce maturation of FDC networks within the nascent follicle.

The importance of LTα in lymphoid organogenesis was first realized when $LT\alpha^{-/-}$ mice were generated and found to have no lymph nodes or Peyer's patches, and chaotic splenic white pulp in which segregation of T and B cells is absent [32, 33]. Subsequently, it was shown that $LT\beta R^{-/-}$ mice also lack all peripheral lymph nodes, Peyer's patches and aggregates of mucosa-associated lymphoid tissue [34]. It is now clear that interactions between $LT\alpha_1\beta_2$ and LTβR are crucial for lymph

node development, as well as formation of Peyer's patches and normal splenic white pulp architecture.

Studies using reciprocal bone marrow chimeras have helped to delineate the roles of hematopoietic and mesenchymal cells bearing these receptor-ligand pairs during organogenesis. When wild type recipients are reconstituted with $LT\alpha^{-/-}$-derived bone marrow, lymphocytes segregate normally within lymph nodes. By contrast, when wild type bone marrow is used to reconstitute irradiated $LT\alpha^{-/-}$ recipients after embryogenesis is complete, secondary lymphoid architecture remains indistinguishable from that of $LT\alpha^{-/-}$ mice [35]. The conclusion from these and other studies is that the crucial interaction during lymph node organogenesis takes place between LTβR on mesenchyme-derived stroma and $LT\alpha_1\beta_2$ on bone marrow-derived (hematopoietic) cells. Aggregate morula chimera studies have confirmed that LTα on hematopoietic cells interacts with LTβR on

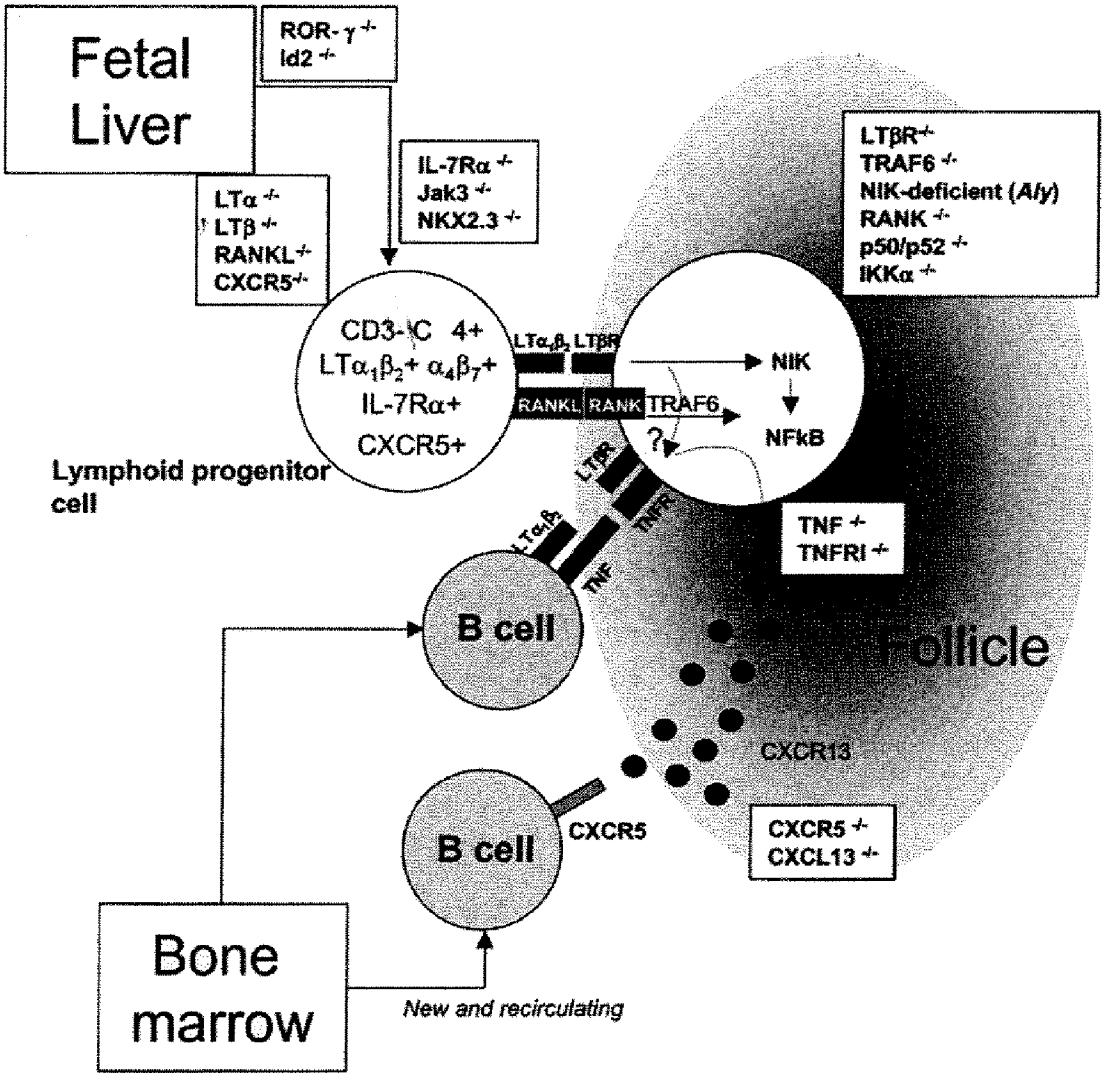

Figure (5). Lymphoid organogenesis.

$\alpha_4\beta_7^+$ fetal liver-derived lymphoid progenitor cells bind to MAdCAM-1 on lymph node high endothelial venules. Ligation of LTβR and RANK on mesenchymal cells stimulates secretion of chemokines including CXCL13, which upregulates $LT\alpha_1\beta_2$ expression on lymphoid progenitors. Activation of mesenchymal cells via the LTβR involves signaling through NIK. Signaling downstream of RANK involves TRAF6, and NF-κB may be activated downstream of both pathways. Under the influence of CXCL13, B cells are attracted to the follicles. Expression of $LT\alpha_1\beta_2$ and TNF by B cells induces maturation of the FDC network. Mutant mice that have provided evidence for this scheme are included in the boxes.

mesenchymal cells during embryogenesis. When bone marrow-derived cells express LTα, normal lymphoid structure is established even if the stroma is LTα-deficient [36].

Recent evidence suggests that interactions between RANK (receptor activating NF-κB), another TNF-family member, (also known as TRANCE and osteoprogenerin ligand (OPGL)) and RANK ligand regulate homing of $CD45^+$ $CD4^+$ $CD3^-$ $\alpha_4\beta_7^+$ $LT\beta^+$ $CXCR5^+$ lymphoid progenitor cells to nascent lymph nodes [37]. RANK-deficiency results in the complete absence of lymph nodes, while the splenic white pulp is normal. Peyer's patches are smaller than normal but their architecture is preserved [37-39].

Interactions between $LT\alpha_1\beta_2$ on progenitor cells and LTβR on mesenchymal cells result in a positive feedback loop of mutual activation, liberating chemokines that attract other bone marrow derived cells, including B cells. Progenitor cells express mRNA for CXCR5, and it is likely that mesenchymal cells release chemokines including CXCL13 that augment $LT\alpha_1\beta_2$ expression, Fig. (**5**) [40] (*Section 3.2*).

$LT\alpha_1\beta_2$ binding to LTβR may not account for all lymphoid organogenesis. Blockade of the ligand for LTβR with dimeric soluble LTβR-human IgG1 fusion proteins during gestation, results in near total abrogation of lymph node and Peyer's patch development in the fetus [41]. Since complete blockade of all lymph node development is obtained by the addition of TNFRI-Ig [42], it is possible that ligation of TNFRI (by $LT\alpha_3$) also contributes to mucosal lymphogenesis. Ligation of $LT\alpha_3$ may also play a role in lymph node development, since $LT\beta^{-/-}$ mice often have one prominent mesenteric lymph node and cervical lymph nodes are present, indicating an incomplete defect in lymphoid organogenesis. Thus, $LT\alpha_1\beta_2$ binding to LTβR does not seem to account for all lymphoid organogenesis, as mice doubly-deficient in TNFRI and LTβ do lack all lymph nodes [43, 44]. This might indicate that $LT\alpha_3$ contributes to mesenteric lymph node and some peripheral lymph node development. Alternatively, incomplete absence of lymph nodes in $LT\beta^{-/-}$ mice may be because $LT\alpha_1\beta_2$ is not the only ligand for LTβR during lymphoid development.

Signaling During Organogenesis

Some of the signaling pathways downstream of TNFR family members that operate during lymphoid organogenesis have been elucidated. The natural mutant alymphoplasia (*aly*) strain of mice has no lymph nodes or Peyer's patches and has disordered splenic white pulp, a phenotype very similar to that of $LT\alpha^{-/-}$ and $LT\beta R^{-/-}$ mice [45]. Aggregate morula chimera studies have shown that wild type bone marrow cannot correct the phenotype when the stroma is derived from *aly* mice [36]. This is the opposite of the result obtained with $LT\alpha^{-/-}$ chimeras and indicates that the *aly* gene product acts on the mesenchymal side of the ligand interaction. This conclusion has been borne out by recent evidence that *aly* mice carry a mutation of NF-κB-inducing kinase (NIK) [46, 47], which is normally activated after ligation of LTβR.

NF-κB (p50/p52) and I-κB participate in the signaling pathways downstream of TNF receptors. In resting cells, I-κB molecules associate with the NF-κB complex, which is retained in the cytoplasm in an inactive state. Activation of I-κB kinase phosphorylates and degrades I-κB, resulting in its dissociation from NF-κB, which then translocates to the nucleus as an active molecule [49]. Both NIK and inhibitor of I-kB kinase (IKK) are involved in activation of NF-κB. Predictably, disruption of the genes encoding for a number of these molecules results in disordered lymphoid development. p50/p52 double knockout mice lack lymph nodes [48]. IKKα-deficiency is lethal, but day 18 IKKα embryos lack Peyer's patches and fetal liver chimeras generated with $IKK\alpha^{-/-}$ donors exhibit disrupted follicles and impaired B cell maturation [50] [51]. It is likely that IKK is involved in lymph node development. I-κB and NF-κB are also involved in lymphoid organization, follicular development and germinal center formation rather than organogenesis and will be considered below (*Section 3.1*).

Signaling pathways downstream of RANK should leave Peyer's patch development intact. TRAF6 binds to the cytoplasmic domains of CD40, IL-1 receptor and RANK and links these molecules with NF-kB activation. TRAF6 does not bind to LTβR. TRAF-6 deficiency results in lymph node deficiency and splenomegaly, as well as osteopetrosis, while Peyer's patches remain almost normal [52].

2.3 Spleen Development

The spleen arises from the dorsal mesogastrium, a mesodermal precursor of adult mesentery. In mice, splenic development is specifically abrogated by deficiency of the orphan homeobox 11 gene (Hox11). Although $Hox11^{-/-}$ mice are asplenic, they have no other recognizable abnormality [53]. Indeed, the pancreas is normal even though it also arises from the dorsal mesogastrium, indicating that Hox11 only affects the mesodermal cells within the mesogastrium that are destined to form the spleen. Isolated absence of the spleen is only present in about 10% of asplenic humans and analogous mutations have not been reported. More commonly, asplenia is associated with cardiac, pulmonary, hepatic and craniofacial defects [54].

Other gene defects have been identified that result in murine asplenia. The NK homeobox family member Bapx1 is an early developmental marker

for splanchnic mesoderm, consistent with its putative role in visceral mesoderm specification. Deficiency of Bapx1 results in asplenia as well as abnormal development of the ventromedial structure of the vertebral column, some of the craniofacial bones, and gastroduodenal malformation [55]. Capsulin is a basic helix-loop-helix transcription factor that appears to act after splenic specification to control morphogenetic expansion of the splenic anlage via interactions with Hox11 and Bapx1. Capsulin-deficient mice are asplenic [56].

Disruption of homeodomain NKX2-3 results in splenic atrophy and disorganization of the splenic white pulp that remains. NKX2-3 regulates MAdCAM-1 expression within the spleen (and gut). The splenic phenotype of NKX2-3$^{-/-}$ mice may reflect the importance of MAdCAM-1 - $\alpha_4\beta_7$ interactions for normal development of the white pulp. This proposition emerged from the finding that administration of $\alpha_4\beta_7$ blocking antibodies to pregnant mice caused similar splenic white pulp disruption [23]. By contrast, administration of MAdCAM-1 blocking antibodies to adult mice does not appear to affect splenic organization [57].

As with lymph node organogenesis, normal development of splenic white pulp depends on LTβR ligation and the white pulp is severely disrupted in LTα$^{-/-}$, LTβ$^{-/-}$, and LTβR$^{-/-}$ mice. Furthermore, post-natal blockade of LTβR via expression of a LTβR-IgG1 transgene or administration of blocking antibodies results in splenic white pulp disorganization, indicating that LTβR ligation is also necessary to maintain white pulp architecture once it has been established [58-60].

3. ORGANIZATION OF B CELL FOLLICLES

3.1 Follicle Development

Follicle development exemplifies the second phase of lymphoid organogenesis in which lymphocytes are recruited (B cells), and induce maturation of mesenchymal elements (follicular dendritic cells, FDCs) within the nascent organ, Fig. (**4**). Mature recirculating B cells are the predominant cells within lymphoid follicles. Follicles also contain FDCs derived from local mesenchymal cells [61], which are thought to play an important role as an antigen depot within follicles.

Normal follicular development is closely related to maturation of the FDC network. LTβR and TNFRI are expressed by FDCs, and both receptors need to be ligated in order to induce a normal FDC network and follicle formation. Bone marrow chimera studies have established that expression of TNF, LTα and LTβ by hematopoietic cells induces FDC maturation and normal follicular architecture in LTα$^{-/-}$ and TNF$^{-/-}$ recipients [62, 63]. Indeed, purified wild-type B cells are sufficient to restore FDC, follicles and germinal center reactions to LTα$^{-/-}$ recipients. Furthermore, LTα expression by B cells appears to be essential for B cells to populate follicles, and this reflects the importance of LTα for induction of intrafollicular chemokine secretion (*Section 3.2*), Fig (**5**).

Lymph nodes are present in both TNF$^{-/-}$ and TNFR$^{-/-}$ mice but mature FDC networks are absent and follicles do not form normally; segregation of T and B cells in splenic white pulp is incomplete [63, 64]. Instead of follicles, B cells form homogenous rims between the periarteriolar T cell zone and the red pulp. When assessed by flow cytometry, both marginal zone and follicular B cell populations are present in normal numbers in TNF$^{-/-}$ mice, indicating that both B cell populations are located within this region [65, 66].

Lymphoid architectural abnormalities like those observed in TNF$^{-/-}$ and TNFRI$^{-/-}$ mice have also been observed in mice bearing other genetic defects. NF-κB2/ p52$^{-/-}$ mice appear to be identical to TNF$^{-/-}$ and TNFRI$^{-/-}$ mice, with abnormal follicles and absence of mature FDC networks and metallophillic macrophages [67, 68]. A similar but incomplete defect is also observed in mice lacking Bcl-3, an I-κB-related regulator. In Bcl-3$^{-/-}$ mice, FDC networks are observed after immunization. Transfer of normal bone marrow into NF-κB2/ p52$^{-/-}$ recipients restores metallophillic macrophages but not FDC networks. The similarities between TNF$^{-/-}$ (and TNFRI$^{-/-}$) and NF-κB2/ p52$^{-/-}$ mice reflect the failure of FDCs in NF-κB2/ p52$^{-/-}$ mice to express the TNF-dependent chemokine, CXCL13 [68].

3.2 Chemokine Regulation of B Cell Migration

Chemokines are a family of small soluble molecules that are unified by the capacity to bind to 7-transmembrane-spanning receptors that are coupled with cytoplasmic heterotrimeric G-proteins. These interactions are complex, since chemokines have pleiotropic functions, and there is promiscuity in ligand binding by receptors. Furthermore, different combinations of G-protein subunits mediate linkage with different intracellular signaling cascades. Finally, chemotaxis is not mediated exclusively by chemokine receptors, and the cross-talk between signaling activated by stimulation of non-chemokine receptors and chemokine receptors remains uncertain.

In general terms, lymphocyte chemotaxis appears to be guided by fluctuations in chemokine concentration gradients within different microenvironments [69]. Regulation of lymphocyte migration, homeostasis and immune responses by chemokines has been reviewed elsewhere [70, 71].

The discussion here is confined to their role in regulating B cell movement.

Although TNF and LTα must be expressed on B cells during development in order for normal follicles to form, when $TNF^{-/-}$ and $LT\alpha^{-/-}$ B cells are injected into normal mice, they migrate normally into established follicles [72]. This is because expression of TNF and LTα by B cells is necessary to establish follicles, and this includes production of follicle specific chemokines. Thus, follicle formation depends on TNF and LT binding to their receptors, but this ligand does not mediate follicular homing directly. Instead, these interactions induce follicular mesenchyme to secrete chemokines that attract B cells.

Naive B cells localize within B cell follicles under the influence of CXCL13 (also known as B lymphocyte chemoattractant, BLC, or B cell attracting chemokine-1, BCA-1). The receptor for CXCL13 is CXCR5 (or Burkitt's lymphoma receptor (BLR)-1), which is highly expressed on B cells. FDCs and their immature stromal precursors have been shown to produce CXCL13 after stimulation by $LT\alpha_1\beta_2^+$ cells [27]. Interestingly, there is a reciprocal interaction between B cells and FDCs because in the absence of CXCL13, $LT\alpha_1\beta_2$ is not upregulated on naïve B cells and consequently FDCs fail to mature [27]. Both $CXCL13^{-/-}$ and $CXCR5^{-/-}$ mice have abnormal splenic white pulp and Peyer's patches, and apart from inguinal and mucosal groups, lymph nodes are absent.

Ligation of LTβR stimulates CXCL13 secretion but TNFRI ligation is also required [40]. However, the splenic white pulp of $CXCR5^{-/-}$ mice resembles that of $TNF^{-/-}$ mice rather than $LT\beta R^{-/-}$ mice [73]. This suggests that LTβR ligation during organogenesis is necessary to allow TNFRI ligation to induce CXCL13 production. The failure of TNF-blockade to disrupt follicular structure suggests that CXCL13 secretion is independent of TNF after organogenesis is complete, Fig. (**5**).

When naïve CXCR5-deficient B cells are transferred into normal recipients, they locate in the T zones but fail to enter the follicles [73]. A similar pattern of migration is observed after stimulation with antigen when B cells migrate to the outer T zones of spleen and lymph nodes (*Section 5.2*). This change in migration pattern is regulated by upregulation of CCR7, a receptor for CCL19 (ELC) and CCL21 (SLC), which are both expressed in the T zones [74]. Location in the outer zone reflects balanced attraction to T zone and follicular chemokines.

Interactions between lymphocytes and adhesion molecules expressed on high endothelial venules within secondary lymphoid organs, or in sites of inflammation, are thought to play an important role in enabling lymphocytes to exit the intravascular space and enter the parenchyma. MAdCAM-1 is expressed by high endothelial venules of Peyer's patches and mesenteric lymph nodes and its expression depends on ligation of TNFRI and signaling involving NF-κB. MAdCAM-1 appears to play a crucial role in lymphocyte homing to Peyer's patches and mesenteric lymph nodes (*Section 2*). However, neither anti-MAdCAM-1 treatment nor blockade of its ligand $\alpha_4\beta_7$ influence B cell homing to the white pulp of the spleen even though it is expressed on the marginal sinus endothelium [57]. An alternative mechanism for lymphocyte entry into the white pulp has been proposed in which B cell location in the white pulp is determined by binding to marginal zone macrophages adjacent to the marginal sinus [75]. Consistent with this, fucoidin, which blocks lymphocyte binding to marginal zone macrophages, blocks lymphocyte entry into the splenic white pulp. By contrast, pertussis toxin, which blocks $G\alpha_i$-coupled receptor signaling, does not affect B cell entry in the spleen, but blocks responsiveness to chemokines and causes aberrant B cell positioning in the white pulp [76].

4. B CELL RECEPTOR SENSITIVITY, REPERTOIRE SELECTION AND COMPARTMENTALIZATION OF B CELLS

A remarkable feature of B cell biology is that many elements of the B cell response are determined by signals through the B cell receptor (BCR), including selection and survival within the recirculating follicular repertoire, location in secondary lymphoid tissue, tolerance of self-reactive B cells, and responsiveness to antigen. It is therefore not surprising that ligation of the BCR activates many intracellular signaling pathways, which ultimately modulate survival versus apoptosis, cell cycle progression, and differentiation. Mutations of the BCR or B cell co-receptors and their downstream signaling pathways not only affect B cell homeostasis and regulation of different B cell subsets, but also change BCR signaling thresholds and these changes may be associated with autoimmune or immunodeficiency phenotypes.

4.1 B Cell Receptor and Co-receptors: Implications for Migration and Responsiveness

The BCR consists of membrane Ig (mIg), which contains a short intracytoplasmic tail non-covalently linked to Igα and Igβ (CD79a and CD79b) [77]. These molecules initiate generic signaling after BCR ligation. Nevertheless, the signal is influenced by the nature of the Ig cytoplasmic tail. Naïve B cells express membrane IgM and IgD and after stimulation with antigen, B cells may undergo switching to downstream Ig isotypes (IgA, IgG, and IgE). The conserved C-terminal sequences of the cytolasmic tails of switched mIg molecules has been shown to

mediate antigen presentation and thus regulate and enhance the expansion and differentiation of memory B cells [78-81].

The signaling subunits of CD79a and CD79b contain immunoreceptor tyrosine-based activation motifs (ITAMs), which are activated primarily by Src-related protein tyrosine kinases after BCR cross-linking by antigen, and provide crucial links with downstream protein tyrosine kinases. Src-kinases accumulate in glycolipid-enriched microdomains (lipid rafts), which are also rich in related kinases, while negative regulators are excluded [82]. After ligation of antigen, BCRs translocate into lipid rafts and this environment facilitates efficient ITAM activation [83].

Several B cell surface molecules are associated with the BCR and modulate BCR signaling. These co-receptors are either associated constitutively with the BCR, or become associated with it after ligation with antigen and they alter the threshold for B cell activation. Evidence from mice in which B cell co-receptor function has been modulated suggests that they might be important for pathogenesis of B cell-mediated diseases.

Co-receptors can be classified according to whether they increase or decrease the threshold for B cell activation after ligation of the BCR (Table I). Those that increase the threshold dampen antibody responses, while those that decrease the BCR signaling threshold increase antibody responses. Thus, an increase in BCR signaling threshold may result in immunodeficiency, while a decreased BCR threshold may result in autoimmunity.

a. Negative Regulators

Negative regulators of BCR signaling include CD22, CD72 and FcγRIIB1. The cytoplasmic tails of CD22, CD72 and FcγRIIB1 contain immunoreceptor tyrosine based inhibition motifs (ITIMs), which increase the BCR activation threshold by recruiting phosphatases that down-modulate BCR signaling. CD22 and CD72 are constitutively associated with the BCR, while FcγRIIB1 associates with the BCR after ligation by Fc regions of Ig within circulating immune complexes. CD22 may be ligated by α2-6-sialylated moieties on CD45RO, CD22 itself [84], and possibly other unidentified ligands. CD72 binds the class IV semaphorin CD100, which is expressed on B cells and activated T cells [85, 86] and CD5 [87], which is present on B-1 cells and at high levels on T cells.

CD22 and CD72 recruit SH2-containing protein tyrosine phosphatase (SHP)-1 and FcγRIIB1 recruits SH2-containing inositol polyphosphate 5-phosphatase (SHIP). Activation of both phosphatases leads to reduced calcium mobilization in response to BCR ligation [88, 89]. The tyrosine kinase Lyn, which is activated after BCR ligation, phosphorylates CD22 and FcγRIIB1 [90, 91]. Although Lyn is also thought to phosphorylate CD79a/b ITAMs, studies with mutant mice suggest that its net effect is negative regulation. Mice deficient in either CD22, SHP-1, FcγRIIB1, or Lyn all exhibit a similar phenotype of B cell hyper-responsiveness, and this is associated with immunopathology (Table II, *Section 6*) [92, 93]. Furthermore, the interaction between these negative regulators helps to explain why mice with

Table I. B cell coreceptors and signaling thresholds.

Deficiency	BCR threshold	B-1	Follicle	MZ	TD	TI-2	References
SHP-1	↓	↑	↓				[270]
Lyn	↓	↑	↓		N	↓	[271]
FcγRIIB1	↓	N	N	N	↑	↑	[93, 272]
CD22	↓	↓			N	↓	[731, 274]
CD72	↓	↓	↓	N	↑	↑	[275]
Aiolos	↓		↑	↓	↑		[95, 122]
CD19	↑	A		A	↓	N	[134, 111]
CD21	↑	↓		N	↓		[190, 191]
CD45	↑				↓		[123]
Vav-1	↑	↓			↓	N	[276]
Vav-1/2	↑		↓		↓	↓	[277]
Btk	↑	↓	↓	N	N	↓	[95, 219]
Syk	↑	↓	↓		↓	↓	[121]

N = normal; A = Absent

Table II. B Cell receptor signaling threshold and autoimmunity.

Defect	Autoantibodies	Reference
$Fc\gamma RIIB^{-/-}$	Antinuclear antibodies Anti-glomerular basement membrane antibodies Antinuclear antibodies	[93] [272] [273]
$Lyn^{-/-}$	Antinuclear antibodies Glomerulonephritis	[91] [92]
$SHP\text{-}1^{-/-}$	Hypergammaglobulinemia Autoantibodies dsDNA antibodies Glomerulonephritis	[270]
$CD45^{-/-}$	Antinuclear antibodies	[123]
$CD22^{-/-}$	dsDNA antibodies Cardiolipin antibodies Myeloperoxidase antibodies (Antineutrophil cytoplasmic antibodies)	[273]
CD19 Tg	Antinuclear antibodies Rheumatoid factor dsDNA antibodies	[278]

heterozygous defects in Lyn, CD22 and SHP-1 exhibit a hyper-responsive phenotype similar to $Lyn^{-/-}$ mice [94].

Recent studies have identified Aiolos as another important negative B cell regulator, since $Aiolos^{-/-}$ mice exhibit an activated cell surface phenotype and undergo augmented BCR-mediated *in vitro* proliferation responses, even with limiting amounts of antigen [95]. The role of Aiolos is complex and is discussed below in relation to recruitment of B cells into other compartments. *In vivo*, $Aiolos^{-/-}$ B cells are activated and form germinal centers even in the absence of immunization, and there is evidence of immune-mediated inflammation and an increased incidence of lymphoma.

b. Positive Regulators

CD45 is a membrane-bound tyrosine phosphatase, which is expressed in different isoforms in a cell-specific manner. B cells and double negative thymocytes express the largest molecular form of CD45 (CD45R/B220). CD45 plays an essential role in B cell activation: After BCR stimulation in $CD45^{-/-}$ mice calcium mobilization is impaired, and there are defects in activation of multiple downstream signaling pathways. In the absence of CD45, CD40-mediated signals are not affected, and T-independent signals to viral antigens remain intact [96]. CD19, CD21, CD81 (TAPA-1), leu13 and γ-glutamyl transpeptidase form a co-receptor complex that decreases the threshold of B cell activation. Consequently, deficiency of these molecules results in impairment of antibody responses (Table I). In addition, this co-receptor complex provides an important link with the innate immune response, because the C3d complement degradation product binds CD21 [97]. This helps explain how complement-coated antigens enhance B cell responsiveness, since ligation of CD21 recruits CD19 into the BCR complex, which in turn enhances downstream calcium fluxes and phosphorylation of Src-family kinases.

Most mice bearing mutations of positive coreceptors exhibit diminished B cell responses to immunization. However, the role of CD19 is complicated because CD19 also activates the CD22/SHP-1 inhibitory pathway, which then acts in a negative feedback loop to negatively regulate CD19 signaling [98]. Furthermore, CD19 function can be modulated by other co-receptors including CD38, an ectoenzyme that appears to enhance BCR signaling. Consequently, $CD38^{-/-}$ mice have reduced antibody responses to protein antigens (although their phenotype is less severe than that of $CD19^{-/-}$ mice) [95, 99].

4.2 B Cell Selection into Different Compartments

a. Follicular B Cell Selection: Tonic Versus Antigen-mediated Signaling

Estimates of immature B cell turnover indicate there are sufficient bone marrow emigrants to replace the recirculating population every 4-5 days [100]. However, the average life-span of recirculating B cells is six weeks. Thus, the number

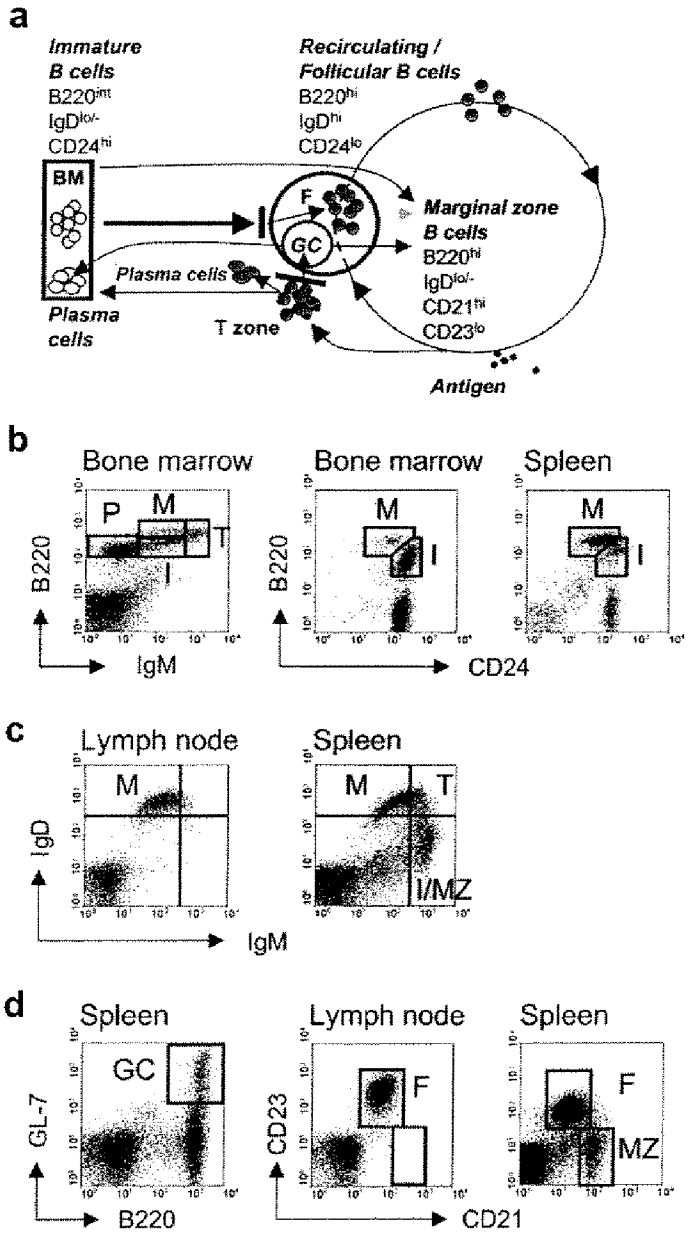

Figure (6). B cell recirculation and compartmentalization.

(a). Summary of B cell recirculation. New bone marrow emigrants compete for entry into the recirculating population that migrates continuously between follicles until stimulated with antigen. This cause arrest in the outer T zone. T cell help leads to differentiation into germinal centers (GC) or plasma cells. Germinal centers give rise to memory B cells and plasma cells. The marginal zone is heterogenous and contains new bone marrow emigrants, memory B cells, and B cells derived from the recirculating population. (b). Surface phenotype of murine B cell subsets determined by flow cytometry. During development, B cell precursors (P) are $B220^{lo}$ IgM^{-}. They express IgM as immature B cells (I) and are $CD24^{hi}$. IgM and B220 are upregulated when they become transitional cells (T) that migrate to the periphery. Mature follicular B cells (M) are IgM^{int} $B220^{hi}$ $CD24^{int}$. (c). The spleen contains IgM^{hi} IgD^{hi} and IgM^{hi} IgD^{lo} B cells, which are transitional and marginal zone B cells. By contrast, lymph nodes contain only mature recirculating follicular B cells (IgM^{int} IgD^{hi}). (d). Murine germinal center B cells (GC) can be identified using GL-7. Marginal zone B cells (MZ) can be distinguished from follicular B cells (F) according to expression of CD21 and CD23.

of recent bone marrow emigrants exceeds the requirement to maintain the follicular population in steady state. Consequently, when the recirculating population is reconstituted from transplanted bone marrow after sub-lethal irradiation of recipients, the fraction of bone marrow emigrants that enter the recirculating population increases [101]. Conversely, when mature B cells are transferred into immunodeficient recipients that lack normal B lymphopoiesis, they survive for much longer than 6 weeks [102]. It follows that a selection event must take place in order for bone marrow emigrants to become incorporated into the recirculating population, and that there is competition between bone marrow emigrants and established follicular cells for places in the recirculating population, Fig. (**6**) [103].

Before considering the basis of B cell selection, it is important to reiterate that the repertoire of BCRs is generated stochastically by gene recombination. Consequently, some BCRs will be specific for self-antigens and activation of these B cells could lead to the production of autoantibodies. However, B cells expressing receptors reactive with self-antigen undergo stringent negative selection by deletion and receptor editing during development in the bone marrow [104-107]. Consequently, most recent bone marrow emigrants that arrive in the periphery are considered to be antigen-naïve and must compete for entry into the recirculating repertoire.

Recent bone marrow emigrants in the periphery are called transitional B cells, identified by the phenotype IgM^{hi} IgD^{+} $CD23^{int}$ $CD21^{int\text{-}hi}$ $B220^{int}$ 493^{+} [108, 109]. It appears that these cells have the capacity to be recruited into follicles (and marginal zones) [110-112]. Irrespective of the nature of the selection event, the population of recirculating follicular B cells is considered antigen-naïve. Selection into the recirculating population seems to be mediated by BCR ligation, since mice with impaired BCR signaling (lacking the Igα cytoplasmic tail) have a severe block in the transition from the immature to the mature B cell pool [113]. Nevertheless, this BCR-mediated selection event must be different from B cell activation by antigen when BCR initiates recruitment of B cells into an immune response. It is possible that this difference hinges on the intensity of BCR stimulus, with weak (subthreshold) stimuli leading to selection while activation involves a stronger stimulus. In this case, selection should be affected by BCR signaling threshold, and the repertoire of recirculating B cells might reflect the BCR ligands available during selection. Possible ligands that might provide this selection stimulus include environmental antigens, self-antigen, or Ig idiotypes.

Analysis of the Ig V_H loci expressed in mature B cells reveals that they differ from those expressed by B cell precursors, consistent with the proposition that antigen specificity influences selection of B cells [114-117]. The fate of B cells specific for self-antigen has been investigated thoroughly in mice that express both a transgene-encoded BCR specific for hen egg lysozyme (anti-HEL) and a transgene encoding HEL (as a neo-self antigen). When B cells bind cognate self-antigen with low avidity they enter the follicles as anergic B cells [118], which have a shortened life span and are hyporesponsive to subsequent BCR ligation. This represents one situation in which antigen ligation has been demonstrated conclusively to occur before B cells enter the follicular population. As such, anergic B cells are not naïve recirculating follicular B cells. Furthermore, when self-reactive anti-HEL Ig transgenic B cells represent a minority population within a normal B cell repertoire, they locate in the T zone and fail to enter the follicles. This may indicate that autoreactive B cells compete less effectively than naïve B cells for places in the recirculating repertoire [119]. An alternative explanation is that when there are few antigen-specific B cells in the repertoire, the concentration of antigen per cell pushes the stimulus into the suprathreshold range causing the B cells to migrate to the T zone [120] (*Section 5.2*).

If selection into the recirculating follicular repertoire is BCR-mediated, the proportion of cells selected should be influenced by the BCR activation threshold. There is evidence that this is the case. For example, Syk-deficient B cells have a defect in signaling downstream of the BCR and immature syk-deficient B cells arrest in outer T zones but fail to undergo selection into the mature follicular population [121]. Similarly, mice deficient in either Btk or BLNK, which signals downstream of Syk, have reduced numbers of mature B cells. By contrast, Aiolos-deficiency reduces the BCR signaling threshold and results in increased mature B cells [122]. When the BCR signaling threshold is increased by CD45 disruption, self-antigen selects self-reactive B cells to enter the follicles in preference to non-transgenic B cells [123]. By contrast, when BCR signaling threshold is decreased, self-reactive B cells are deleted before they enter the follicles [124].

Non-self-reactive recent bone marrow emigrants are IgM^{hi} and IgD^{hi}. After selection into the recirculating population, surface IgM undergoes variable downregulation. Thus, follicular B cells are IgD^{hi} and express low to intermediate levels of IgM, (and anergic B cells, which are definitely antigen-experienced, are IgD^{hi} IgM^{lo}), Fig. (**6**). Stimulation of B cells with antigen *in vivo* causes a reduction in the level of IgM expression in proportion to the intensity of the stimulus [120]. Downregulation of IgM on follicular B cells therefore, may represent further evidence of a subthreshold BCR ligation during follicular selection. Consistent with this idea, in $Aiolos^{-/-}$ mice there is increased B cell proliferation in response to BCR ligation and

therefore the threshold for B cell activation is lowered [95] and most of the peripheral B cell repertoire consists of mature IgD^{hi} IgM^{lo} follicular B cells [122].

The evidence for ligand mediated-selection is circumstantial. An alternative hypothesis of B cell selection suggests that BCR signaling occurs in the absence of ligation with antigen because receptor oligomerization occurs in resting B cells, which leads to signal transduction [125]. According to this model, the BCR transmits a signal constitutively, and this tonic signal is modified when the BCR itself is ligated, which would cause disruption of the oligomerized receptor complex [126]. A functional BCR would therefore generate a selection and survival signal in the absence of antigen. This model could also accommodate the inverse relation between mIgM expression and BCR signaling threshold, since in the presence of lowered threshold, a high density of mIgM would be required to maintain the selection signal. This may explain why abrogation of BCR expression curtails the B cell life-span [127]. On the other hand, evidence has been presented that it is the V region of the BCR rather than the BCR *per se* that is crucial for B cell competition and survival in the periphery [128].

b. B-1 Cell Selection

B-1 cells are a population of self-replicating B cells whose ontogeny remains contentious [129]. B-1 cells dominate the neonatal repertoire. They are responsible for production of natural (IgM) antibodies that bind antigen with low affinity. The importance of these antibodies for host defense remains uncertain, B-1 cells represent only a very small minority of the adult peripheral B cell repertoire, and most peripheral B-1 cells are located in the marginal zone of the spleen. Nevertheless, even in adults there is a significant population of B-1 cells on the serosal surfaces such as the peritoneum [130].

It has been suggested that B-1 and (conventional) B-2 cells belong to separate lineages, however, recent evidence suggests that these subsets may be selected by BCR ligation. Mature splenic B-2 cells can give rise to peritoneal B-1 cells after adoptive transfer and there is a close correlation between BCR activation threshold and selection of B-1 cells (Table I) [131, 132]. A decrease in the BCR activation threshold leads to an expansion of B-1 cells, while mutations that increase B cell activation threshold abolish the B-1 population [124, 133, 134]. B-1 cells are selectively absent from both Btk-mutant and $CD19^{-/-}$ mice, as well as from mice bearing other mutations that affect the Btk signaling pathway. Conversely, the population is increased in $CD22^{-/-}$, $CD72^{-/-}$, $Lyn^{-/-}$ and $Aiolos^{-/-}$mice where the BCR signaling threshold is decreased. Thus, the size of the B-1 compartment is a surrogate marker of the BCR activation threshold. These data are also consistent with the finding that treatment with Cyclosporin A, which interferes with signaling downstream of the BCR, appears to block selection into the B-1 repertoire [135]. Finally, certain BCR specificities appear to guide the development of B-1 or B-2 B cells [136]. Indeed, strong self-antigen binding appears to be a prerequisite for B-1 selection [137].

c. Marginal Zone B Cell Selection

The ontogeny of marginal zone (MZ) B cells remains to be established with certainty. MZ B cells appear to be a mixture of immature B cells, memory (post-germinal center) B cells, B cells derived from the naive recirculating follicular population, and B-1 cells. Recent analysis of gene expression by MZ B cells confirms that this population includes cells carrying both germline and somatically mutated Ig V_H regions [138, 139].

MZ B cells are larger, have less condensed chromatin, and express higher levels of costimulatory molecules (CD80 and CD86) than recirculating follicular B cells [140, 141]. These features suggest that MZ B cells are in a state of partial activation, and are consistent with evidence that they can undergo rapid differentiation to plasma cells after ligation with antigen [142]. Other phenotypic features that distinguish this population are high levels of IgM and low levels of IgD, as well as high levels of CD21 expression and low levels of CD23, Fig (**6**).

In contrast to follicular B cell selection, MZ B cell selection may be related with the strength of BCR signaling, because the marginal zone is small in $Aiolos^{-/-}$ mice, where the BCR signaling threshold is decreased. By contrast, the marginal zone is increased in $Btk^{-/-}$ and $CD21^{-/-}$ mice, where the signaling threshold is increased. On the other hand, two strains of mice expressing BCR transgenes have been identified in which B cells are preferentially selected to enter the MZ based on Ig heavy chain expression, raising the possibility of positive selection [111, 143]. Furthermore, selection of these B cells is lost in the absence of Btk. These apparently conflicting results may reflect the heterogeneity of MZ B cells.

Chemokines may also play a role in establishing the MZ. Mice lacking the tyrosine kinase Pyk-2 have a selective marginal zone deficiency [144]. Adoptive transfer studies indicate that Pyk-2 must be expressed in the B cells rather than the stromal components of the spleen in order for MZs to develop [144]. Pyk-2 participates in signaling pathways downstream of chemokine and growth factor receptors but the putative chemokine has not been identified. Administration of pertussis toxin, which blocks signaling through chemokine receptors [76], has also been shown to deplete MZ

B cells [144]. Taken together, these findings imply that MZ B cell organization, like other B cell compartments, depends on secretion of a chemokine by mesenchymal/stromal elements.

The presence of unique macrophage populations (marginal zone macrophages and metallophillic macrophages) within the MZ raises the possibility that they may provide such stromal support. This hypothesis is supported by the identification of situations in which these macrophage populations are defective or absent. MZ metallophils are absent in early life and their appearance coincides with formation of marginal zones [145]. Furthermore, in mice in which LTβR ligation is disrupted, there is no identifiable MZ. Analysis of splenocytes from TNF/LTα$^{-/-}$ mice by flow cytometry has shown that B cells bearing the phenotypic hallmarks of MZ cells are absent [66]. Both MZ macrophages and metallophillic macrophages are also absent from these mice.

Finally, survival may influence MZ B cells. Ligation by BAFF, another TNF family member expressed by monocytes, macrophages, dendritic cells and T cells, appears to be important for maintaining transitional B cells within the MZ. BAFF has been shown to regulate survival of transitional B cells through increasing the levels of Bcl-2 expression [112]. Mice transgenic for BAFF have increased numbers of transitional cells and MZ B cells. BAFF receptors identified so far are BCMA and TACI, but since mice deficient in these receptors form normal marginal zones [146, 147] it is likely that a third BAFF receptor exists on B cells.

4.4 BCR ligation and B Cell Homeostasis

The data discussed so far reveals that signals from the BCR are crucial for locating B cells in different compartments. BCR ligation is also critical for the maintenance of self-tolerance [118], and is obviously critical for responsiveness to antigen. The final aspect of B cell regulation to be considered is that the recirculating B cell population is maintained at a constant size (B cell homeostasis). The question arises as to whether these are independent processes. For example, can B cell homeostasis be maintained independently of follicular selection?

One theory to explain B cell homeostasis is that there is competition for intrafollicular resources, for example a follicle-specific chemokine. This would be analogous to interspecies competition that operates to control population homeostasis in environmental niches. According to this theory, the composition of populations should be altered by manipulation of resources [148]. For example, the follicular chemokine CXCL13 may represent a resource for which B cells compete in order to enter the recirculating population. Although attractive, this theory is at odds with data from TNF-deficient mice because irrespective of the identity of the resource (follicular chemokine(s)), the absence of follicles in TNF$^{-/-}$ mice indicates that the resource is deficient in this strain. Nevertheless, the size of the recirculating follicular population (defined by B cell surface phenotype) is the same in TNF$^{-/-}$ mice and wild type mice, and the B cell life span is normal [66]. These findings suggest that B cell homeostasis is distinct from follicular localization and that follicular chemokines act simply to organize B cells into follicles. This conclusion is also consistent with evidence that maintenance of the follicular population after selection depends on continuous expression of the BCR [127].

Evidence has been presented that BCR sensitivity or the capacity to respond to ligation corresponds with surface IgM expression. In other words, the capacity of B cells to respond to BCR ligation appears to be modulated by prior BCR signaling. Recent bone marrow emigrants that are IgM^{hi}, are most responsive, while anergic cells (IgM^{lo}, IgD^{hi}) are least responsive. Similarly, marginal zone cells that are IgM^{hi} are also very responsive to BCR ligation and readily differentiate into antibody-forming cells [111]. BCR signaling may also mediate homeostasis since the intensity of BCR signaling affects B cell life-span. Anergic B cells, which are IgM^{lo} are short-lived and are lost rapidly from the repertoire. A correlation between life-span and intermediate levels of IgM expression remains to be determined.

5. B CELL RESPONSE TOANTIGEN:SPATIO-TEMPORALANDMOLECULARCONSIDERA-TIONS

5.1 Classification of B Cell Responses to Antigen

Antigens can be classified as thymus (T cell)-dependent or thymus-independent (TI), based on their ability to induce a response in nude (athymic) mice, where T cell help for B cells is absent. Responses to conventional protein antigens are thymus-dependent (TD). Thymus-independent antigens are sub-divided into type 1 (TI-1), such as lipopolysaccharide (LPS, or endotoxin) and type-2 (TI-2), exemplified by polysaccharides. This classification originates from the observation that polysaccharides fail to elicit B cell responses in CBA/N (also known as X-linked immunodeficiency, Xid) mice, even though they respond normally to both TD and TI-1 antigens [149].

An effective B cell response to protein antigen involves B cell migration to the T zones where they interact with antigen-specific primed T cells, proliferate and differentiate. This results in clonal expansion of B cells specific for the antigen that initiated the immune response. There are two main pathways of B cell differentiation. Extrafollicular

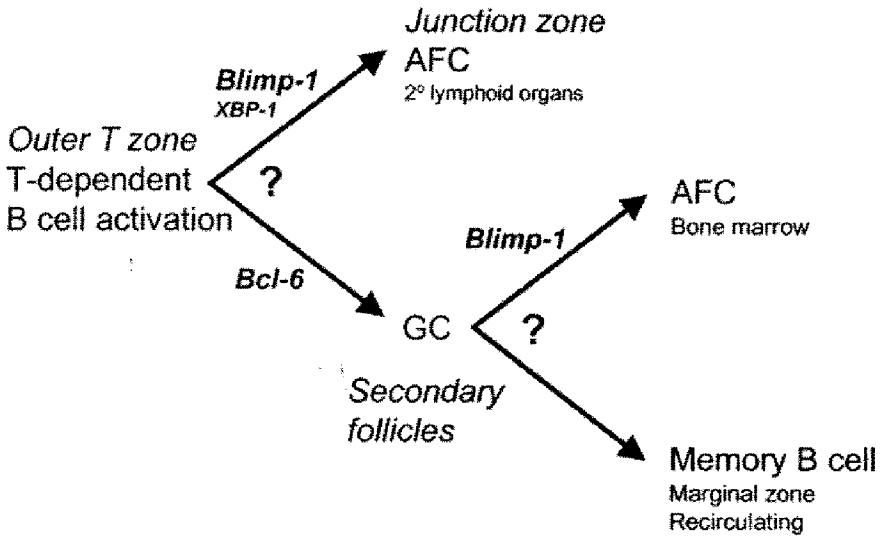

Figure (7). B cell differentiation after immunization.

B cells differentiate into antibody forming cells (AFC) or germinal centers (GC). Germinal center B cells give rise to plasma cells, which migrate to the bone marrow, and memory B cells. GC B cell differentiation is associated with Bcl-6 expression and plasma cell differentiation is associated with Blimp-1 and XBP-1 expression. The signals that determine which pathway of differentiation a B cell will follow remain uncertain.

plasma cells are responsible for early antibody production, whereas intrafollicular proliferation and differentiation leads to germinal centers and formation of high affinity antibody-forming cells and memory B cells, Fig. (7). Recent evidence has identified signals that are necessary for these populations to be established, however, the fundamental basis of the decision to differentiate down one pathway or the other remains unknown. In this section, the key cellular and molecular regulators of B cell migration, activation and differentiation to yield memory or antibody-secreting cells are discussed.

5.2 Early Response to BCR Ligation

A suprathreshold stimulus through the BCR, exemplified by B cell binding to foreign antigen, causes B cells migrate to the outer T zones in secondary lymphoid organs. This pattern of migration is observed whether B cells are stimulated with TD or TI antigen. Even germinal center B cells appear to follow this pattern of migration after BCR ligation if T cell help within the germinal center is not available [150, 151].

The chemokines that guide segregation of resting T and B cells in secondary lymphoid organs also mediate migration of activated B cells. Migration to the outer T zone is mediated by up-regulation of chemokine receptors on B cells, including CCR7, which binds to T zone-derived chemokines such as CCL19 and CCL21. When normal B cells are stimulated with antigen and transferred into TNF/LT$\alpha^{-/-}$ mice, they do not demonstrate any change in their pattern of migration compared with antigen-naïve B cells. CCL19 and CCL21 are relatively deficient in TNF/LT$\alpha^{-/-}$ mice [152]. By contrast, when antigen-stimulated B cells are transferred into TNF$^{-/-}$ recipients, they migrate in the outer T zones. Follicular chemokine production is thought to be defective in these recipients, while the T zones are normal.

5.3 B Cell Activation in the Outer T Zone

After immunization with TD antigen, the fate of B cells in the T zone is determined by the availability of help from primed T cells. Priming takes place when T cells recognize (using their TCR) specific peptide in the context of MHC class II molecules on the surface of interdigitating dendritic cells (IDC). This event usually occurs in the T zones. Once primed, T cells become competent to provide help to those B cells that express the same peptide-MHC complexes. T cell-B cell interactions in the outer T zone are essential for B cell proliferation, isotype switching, and differentiation of B cells into germinal center cells or extrafollicular plasma cells. By contrast, if T cell help is unavailable, as is often the case if a B cell has bound self-antigen, B cells are deleted. Thus, location of antigen-stimulated B cells in the outer T zone is a crucial point for the decision between B cell immunity and tolerance.

By convention, the signals delivered to B cells during activation by protein antigens are divided into two types. Signal 1 is delivered when BCRs are ligated with antigen, and signal 2 is the composite stimulus provided by T cells. This includes the antigen-specific (cognate) interactions between TCR and peptide/MHC on B cells, as well as accessory signals in the form of cell-cell contact and cytokines. Cell-cell contact signals include adhesion molecules such as LFA-1 and ICAM-1, CD40 (*Section 5.3*), and the B7 family of molecules.

CD154 (CD40L) is expressed transiently on activated T cells and ligates CD40, which is expressed constitutively on B cells. This signal is critical for isotype switching and B cell differentiation into both plasma cells and germinal centers after immunization with protein antigens [153-155] (*Sections 5.5 and 5.6*). The B7 family members B7.1 (CD80), B7.2 (CD86) and B7h also influence the B cell response. B7.2 is expressed constitutively on dendritic cells and expression of B7.1 can be induced on activated dendritic cells. Since B7.1 and B7.2 are ligands for CD28 and CTLA4 on T cells, they play a crucial role during T cell priming (by dendritic cells). These molecules also participate in T-B collaboration, but B cells only express B7.1 and B7.2 after activation [156]. B7.2 is upregulated within 24 hours of BCR ligation. B7.1 is induced more slowly after an equivalent stimulus, but is induced efficiently by LPS [157].

$CD28^{-/-}$ and B7.1/B7.2 double-deficient mice exhibit impaired TD B cell responses with complete absence of germinal centers and isotype switching [158, 159]. B7.1 and B7.2 appear to have non-redundant roles during B cell responses, as might be predicted from their different patterns of regulation. $B7.2^{-/-}$ mice have a complete absence of IgG responses and germinal centers after intravenous immunization, whereas these responses are normal in $B7.1^{-/-}$ mice. The defective response in $B7.2^{-/-}$ mice can be overcome by administering antigen in adjuvant. Since adjuvant upregulates B7.1, it can be concluded that B7.1 costimulation compensates for the absence of B7.2 [160].

In contrast with B7.1 and B7.2, B7h is constitutively expressed on B cells and binds the CD28 homologue ICOS (inducible costimulatory molecule), which is expressed on T cells after TCR ligation. B7h expression on B cells is upregulated in response to TNF. LPS can also induce B7h expression in many non-lymphoid tissues [161]. Ligation of ICOS by B7h induces CD154 expression on T cells. $ICOS^{-/-}$ mice exhibit a defect in isotype switching and germinal center formation in response to protein antigen. Interestingly, these mice exhibit a specific defect in T_H2 differentiation and ICOS-deficient T cells produce higher levels of interferon-γ than T cells from wild-type mice, while IL-4 and IL-10 production is reduced substantially. Consequently, IgG1 and IgE switching is severely affected [162, 163].

5.5 Germinal Centers

5.5.1 Overview

Germinal centers are typically, but not exclusively, the result of TD B cell responses. Germinal centers arise from a small number of precursors derived from B cells activated in the T zone, which undergo massive proliferation, giving rise to centroblasts that populate what is known as the dark zone [164, 165]. Centroblasts downregulate surface Ig expression and during proliferation, Ig genes acquire somatic point mutations in a process that appears to be unique to germinal center B cells [6, 166]. Somatic mutations are concentrated in the variable regions of Ig genes (IgV) and change the affinity of the encoded Ig for the immunizing antigen.

Centroblasts exit cell cycle to become centrocytes, which are located in close proximity to mature FDC networks in the germinal center light zone. Centrocytes upregulate somatically mutated Ig, and those that have either lost affinity for the immunizing antigen, or have acquired affinity for a self-antigen are deleted. Rare centrocytes that have acquired IgV mutations that increase their affinity for antigen are selected for survival and differentiation. Selected centrocytes differentiate into long-lived plasma cells [167, 168], which migrate to the bone marrow or lamina propria of the gut, and memory B cells [169], which colonize the marginal zones. Some centrocytes remain in the germinal center and are recycled to become centroblasts again [170].

Somatic mutation in germinal center B cells poses two risks. First, it can lead to emergence of self-reactive B cells and second, introduction of mutations or translocations may transform B cells and lead to malignancy (B cell lymphoma or leukemia). Centrocyte selection is tightly regulated but it is not foolproof. This is illustrated, first, by recent evidence that the majority of B cell malignancies are derived from post-germinal center B cell precursors [171]. Second, in situations where germinal center reactions are stimulated persistently, such as in sites of autoimmune inflammation, the incidence of malignant transformation is increased (*Section 6.6*). Finally, in autoimmune disease, autoantibodies often bind self-antigen with high affinity, and have been shown to contain somatic mutations, indicating that they have arisen from germinal center reactions.

5.5.2 Differentiation of Germinal Center B Cells

a. Extrinsic Signals

Signals delivered during the initiation of TD responses appear to be essential for germinal center

formation. Germinal centers do not form after TD immunization of CD40L$^{-/-}$ mice, or after administration of CD40 blocking antibody at the time of TD immunization (Table III) [155, 172, 173]. CD40 ligation also appears to be important for maintenance of established germinal centers, since administration of CD40 blocking antibody after germinal center reactions are underway results in their dissolution. On the other hand, CD40 ligation alone is not sufficient to induce germinal center B cell differentiation, because B cells stimulated with CD40L *in vitro* differentiate slowly and acquire an unphysiological phenotype.

Blockade of CD86 (B7.2) ligation at the time of B cell activation abrogates germinal center formation but unlike CD40 ligation, blockade of CD86 after germinal centers have formed has no effect [172]. Similarly, germinal centers fail to form in CD28$^{-/-}$ mice [174]. This probably reflects the importance of CD86-CD28 interactions during T cell priming rather than B cell activation. On the other hand, CD86 ligation may also activate T cells destined to migrate into the germinal center. Costimulation through OX40 and CD28 has been shown to upregulate CXCR5 on T cells, suggesting this signal may be crucial during differentiation of T cells to migrate into B cell follicles and promote germinal center formation [175].

Table III. Molecular defects of isotype switching and germinal center formation

	Isotype switching	Germinal centers
B cell intrinsic		
CD40	–	–
CD40L	–	–
CD153	–	++
B7.1/B7.2	–	–
B7h	–	–
LTα	–	–
TNF	++	–
CXCR5	++	E
OBF-1	–	–
Spi-B	+	+ (short-lived)
AID	–	+++
Bcl-6	++	–
I-κB	+	–
B cell-extrinsic		
TNFR	++	–
LTβR	–	E
CD28	++	–
CXCL13	++	E
NF-κBp52	+	–
IL-6	+/–	+/–
NIK	–	–

E = ectopic

b. B Cell Intrinsic Molecules

i. Bcl-6

Bcl-6 is a transcriptional repressor that appears to be a key regulator of germinal center B cell differentiation [176, 177]. In secondary lymphoid organs of humans and mice, bcl-6 expression is confined to germinal center B cells, both centrocytes and centroblasts, and germinal center T cells [178]. In mice deficient in Bcl-6, germinal centers are absent even though primary follicular structure is normal [176, 177]. Since Bcl-6$^{-/-}$ B cells proliferate normally *in vitro* and Bcl-6$^{-/-}$ mice make normal extrafollicular responses, Bcl-6 appears to be a specific regulator of germinal center differentiation. Bcl-6 acts within B cells to inhibit the extrafollicular pathway of B cell differentiation by suppressing Blimp-1 expression (*Section 5.6.2*) [179, 180].

ii. OBF-1

The B cell-specific transcription factor OBF-1 (also known as OCA-B or BOB-1) is a co-activator of the transcription factors Oct-1 and Oct-2, which bind to the Ig promoter. OBF-1$^{-/-}$ mice have a severe reduction in the number of recirculating follicular B cells. OBF-1 is normally upregulated in germinal center B cells under the influence of CD40 ligation and IL-4 [181]. Germinal centers fail to form after immunization of OBF-1$^{-/-}$ mice with TD antigen. Even though switch recombination takes place in these mice, OBF-1 is also essential for expression of downstream Ig isotypes [182]. Bone marrow transplantation studies show that this is due to B cell intrinsic effects, rather than failure of FDC differentiation [181, 183].

iii. Activation-induced cytidine deaminase (AID) and translesion DNA polymerase ζ

The Activation-induced cytidine deaminase (AID) gene belongs to the family of cytidine deaminases and is specifically expressed in germinal center B cells in mice. AID is required for isotype switching and somatic hypermutation in response to protein antigens [184]. Nevertheless, AID-deficient mice have enlarged germinal centers, and although switch transcripts are expressed, no isotype recombination events take place. This phenotype suggests that the germinal center reactions are futile, and fail to generate memory B cells. Any of the DNA enzymes that may be involved in somatic hypermutation could be targets for AID but a likely candidate is the translesion polymerase ζ, which repairs mismatched DNA bases during DNA replication. Polymerase ζ has been found to be upregulated in human B cells undergoing hypermutation of Ig V and Bcl-6 genes

upon BCR engagement and co-culture with activated autologous T cells. Interestingly, its blockade using specific oligonucleotides inhibits the hypermutation process [185].

5.5.3 Positive Selection of Germinal Center B Cells

a. Hematopoietic-mesenchymal Interactions

Germinal center B cell selection can be thought of in Darwinian terms since centroblasts acquire somatic mutations and then centrocytes are selected according to their fitness. In this case, fitness means expression of BCR with high affinity for foreign antigen and low affinity for self-antigen. The prevailing model suggests that centrocyte selection requires two signals. Signal 1 is delivered when centrocytes bind the antigen that initiated the TD B cell response; this antigen is displayed on the surface of follicular dendritic cells (FDC) within germinal centers. Signal 2 is delivered when centrocytes that compete successfully to bind antigen also receive help from T cells within the germinal center [164].

As described in *Section 3*, the interaction between hematopoietic (B cell) and mesenchymal (FDC) elements is also critical during establishment of normal follicles. This follows from the observation that B cell and FDC networks are absent or poorly formed in mice that lack LTα, LTβ, LTβR, TNF or TNFRI. Development of mature FDC networks depends on ligation of LTβR and TNFRI by $LT\alpha_1\beta_2$ and TNF on B cells [186, 187]. Germinal centers do not form in the B cell areas of these mice after immunization with TD antigen, which probably reflects the lack of FDC-derived CXCL13. However, immunization of $LT\alpha^{-/-}$, $LT\beta^{-/-}$ and $LT\beta R^{-/-}$ mice results in accumulation of germinal center B cells (identified by their capacity to bind peanut agglutinin, PNA^+), which locate ectopically in the T zones adjacent to central arterioles and do not contain mature FDC networks. Although germinal center B cell differentiation and affinity maturation appears to take place in $LT\alpha^{-/-}$ mice, this process is less efficient than under normal circumstances [188]. It requires high doses of antigen administered in adjuvant, raising the possibility that FDCs may normally function to provide an available depot of antigen to mediate efficient germinal center selection.

b. Interactions with Complement

One mechanism by which FDC networks might increase the efficiency of germinal center B cell selection is by enabling a crucial interaction between B cells, antigen and complement. It has long been established that when mice are depleted of complement, germinal centers fail to form [189]. FDCs express CD21 and CD35, which are receptors for complement degradation products (types 2 and 3 complement receptors, respectively (CR2 and CR3)). Since complement is activated on the surface of pathogens, complement degradation products help localize antigen to FDC networks. This is not the only role of complement during germinal center reactions. CD21 and CD35 are both expressed by germinal center B cells and co-ligation of BCR with antigen, and CD21 with complement, dramatically reduces the threshold of B cell activation [97]. Predictably, germinal center formation and memory antibody responses are compromised in $CD21/35^{-/-}$ mice [190, 191]. Careful analysis of this model has revealed that CD21/35 expression on FDCs is necessary to maintain B cell memory, while ligation of CD21/35 on germinal center B cells promotes their survival during the germinal center reaction [192].

c. FcγR on FDCs

FDCs can also trap antigen in the form of immune complexes because they express receptors for the Fc portion of Ig (FcγRs). The importance of circulating immune complexes for germinal center reactions has been put to the test using transgenic mice that express membrane Ig but not secreted (circulating) Ig [193]. In these mice, affinity maturation is intact, which probably reflects the importance of complement in trapping antigen on FDCs. However, the effect of absent secreted Ig on functional long-term B cell memory has not been reported.

5.5.4 Negative Selection of Germinal Center B Cells

Within an ongoing germinal center reaction, germinal center B cells undergo apoptosis if they acquire mutations that result in production of self-reactivity or low-affinity antibody. The expression of key regulators of apoptosis has been mapped in germinal center B cell subsets isolated from tonsils and reveals a coordinated response characterized by downregulation of apoptosis inhibitors and upregulation of pro-apoptotic molecules in centrocytes. Selection of germinal center B cells to become antibody-forming cells or memory B cells requires down-regulation of the anti-apoptotic molecules Bcl-2 and Bcl-x_L, since overexpression of Bcl-2 leads to accumulation of low affinity plasma cells in the bone marrow [194]. By contrast, overexpression of Bcl-x_L leads to accumulation of both low affinity plasma cells and memory B cells [195].

Mice bearing natural Fas mutations accumulate self-reactive B cells that have gone through germinal center reactions, suggesting a possible role for Fas in elimination of autoreactive germinal center B cells [196]. A role for Fas-induced cell death is supported by recent evidence showing that germinal centers express a pre-formed death-inducing signaling complex (DISC) formed by Fas, FLICE (Caspase-8/FADD-like IL-1β converting enzyme) and the FLICE inhibitor, $cFLIP_L$ (FADD- like

IL-1β converting enzyme-inhibitory protein). In the absence of CD40L signals, $cFLIP_L$ is lost resulting in the autoproteolytic cleavage of FLICE. Activation of caspase 8 initiates transduction of death signals [197]. This is consistent with *in vitro* studies of human centrocytes, which show that in the absence of CD40 ligation, centrocytes rapidly undergo apoptosis. It is also consistent with the finding that disruption of CD40-CD40L interactions dissolves germinal centers [172] and evidence that centrocyte binding to self-antigen when T cell help is unavailable results in centrocyte death [150].

5.5.5 Involution of Germinal Centers

Experimental models relying on immunization with artificial antigens such as haptenated proteins have revealed that germinal centers undergo spontaneous involution approximately three weeks after immunization. There exists a ceiling for affinity maturation, which might explain why germinal center reactions are self-limited [198]. Another possibility is that germinal centers involute when T cell help is no longer available. This would be consistent with the finding that immunization with polysaccharide antigen can generate massive germinal centers that involute within 24 hours [199]. After immunization with TI-2 antigen, T cell help is not available within the germinal centers.

On the other hand, recent studies using 'real' antigen (ie live virus) indicate that germinal center life-span may extend beyond three months and therefore provide a continuous source of high-affinity antibody forming cells [200]. Recent evidence has identified a role for the Ets family transcription factor Spi-B in determining germinal center longevity. Spi-B is expressed exclusively in lymphoid cells and is critical for normal B cell development and function. Indeed, the expression of Spi-B increases as B cells mature and deficiency of Spi-B has a profound effect on B cell responses to protein antigen [201]. After immunization, Spi-$B^{-/-}$ mice form small germinal centers, which rapidly acquire excessive apoptotic bodies and involute prematurely. Consequently, memory responses are defective.

Taken together, these findings reveal that while many signals that are obligatory for germinal center formation have been identified, important questions remain unresolved about germinal center involution. Once B cells differentiate into germinal center B cells, apoptosis appears to be the default pathway unless they receive T cell help in the form of CD40 ligation after binding antigen displayed on FDCs.

5.6 Extrafollicular Plasma Cell Foci

5.6.1 Overview

Extrafollicular plasma cell formation is the other pathway of B cell differentiation after interaction with T cells in the outer T zone. Extrafollicular responses play a critical role in host defense because they are responsible for early antibody production [202]. Initially these cells secrete IgM, but later, isotype switching takes place and they also secrete IgG [203].

Extrafollicular responses are initiated when B blasts migrate to the junction zones of the spleen (between the T zones and the red pulp, Fig. (**2d**)) or lymph node medullary cords where they differentiate into proliferating, antibody-secreting plasmablasts. There is evidence that survival and differentiation of extrafollicular plasmablasts into non-dividing plasma cells is regulated by a subset of $CD11c^{hi}$ dendritic cells that co-localize with the plasmablasts in the junction zones of the spleen and medullary cords of lymph nodes [204]. Based on evidence from other B cells subsets, it would be predicted that dendritic cells might provide chemokine support for extrafollicular plasma cells, but direct evidence for this hypothesis is not available. Plasmablasts upregulate Syndecan-1 (CD138) and downregulate mIg but contain high levels of cytoplasmic Ig. After approximately five cell divisions, plasmablasts come out of cell cycle and differentiate into plasma cells [205]. The majority of extrafollicular plasma cells are short-lived and survive only 2-3 days before undergoing apoptosis, although approximately 10% appear to survive for much longer in the spleen [205].

5.6.2 Blimp-1

Within B cells, the transcriptional repressor B lymphocyte-induced maturation protein-1 (Blimp-1) seems to be the key molecule that initiates and maintains plasma cell differentiation. It has been proposed that Blimp-1 is a target of Bcl-6, and according to this evidence, germinal center commitment precludes B blasts from plasma cell differentiation [179]. Plasma cell differentiation would therefore be the default pathway followed by cells that fail to upregulate Bcl-6. Blimp-1 is also upregulated in a subset of germinal center B cells; these cells are probably destined to become high affinity plasma cells rather than memory B cells. Plasma cells derived from germinal centers secrete mutated antibody and migrate to the lamina propria of the gut or the bone marrow where they can survive for long periods without dividing [168].

C-myc is a transcription factor downstream of Blimp-1. Inhibition of *c*-myc transcription, which induces a switch from proliferation to differentiation, seems to be necessary but not sufficient for plasma cell differentiation [206]. Blimp-1 also suppresses CIITA (class II transactivator) transcription. CIITA is a co-factor required for MHC class II transcription, and is expressed in all stages of B cell development up until plasma cell differentiation. Consequently, plasma cells cannot make cognate interactions with T cells [207].

5.6.3 BSAP

Differentiation of mature B cells to plasma cells is also controlled by the DNA-binding transcription factor BSAP (B cell specific activator protein) (PAX-5), which is expressed in all stages of B cell development except plasma cells. BSAP overexpression in a mature B cell line reduced Blimp-1 expression and suppressed spontaneous appearance of plasma cells [208].

5.6.4. X-Box-binding Protein-1 (XBP-1)

It has been shown recently that mice in which lymphocytes are deficient in XBP-1, a basic-region leucine zipper protein of the CREB/ATF family of transcription factors, exhibit normal germinal centers but have decreased antibody titers in response to immunization and small numbers of terminally differentiated plasma cells [209]. A highly activated B cell line could also be driven to differentiate into plasma cells when transfected with XBP-1.

5.6.5 Extrinsic Signals

Ligand pairs from the TNF family regulate plasma cell differentiation. CD27 is expressed by B cells and binds CD70 to deliver a signal that appears to be important for terminal differentiation of B cells into plasma cells [210]. By contrast, engagement of CD153 by CD30 inhibits plasma cell differentiation and expression of blimp-1 [211].

Growth of plasma cells depends on the availability of IL-6, which is secreted by a variety of cells including dendritic cells and B cells themselves. Over-expression of IL-6 is associated with plasma cell dyscrasia, polyclonal plasmacytosis, lymphadenopathy, extramedullary hematopoiesis and mesangiocapillary glomerulonephritis (*Section 6.4*). On the other hand, in mice with IL-6 deficiency, pristane-induced plasmacytosis cannot be induced [212] and these mice exhibit defective germinal center formation and isotype switching [213]. Plasmablasts, especially those arising in germinal centers leave the spleen and home to the bone marrow. This traffic seems to be regulated by down-regulation of the chemokine receptors CCR7 and CXCR5 and increased sensitivity to CXCL12 [214, 215].

5.7 Molecular Control and Architectural Correlates of T Cell-independent Immune Responses

5.7.1 Type 1 T Cell Independent Responses

Lipopolysaccharide (LPS) is an essential component of the outer cell wall of gram-negative bacteria. LPS is a powerful stimulant of both the innate and adaptive arms of the immune system, and it is the effects on the innate immune system that dominate the clinical picture of septic shock.

LPS is a thymus-independent type 1 antigen, and large doses of LPS can activate B cells in a polyclonal fashion. Small doses of haptenated-LPS activate only hapten-specific B cells, and LPS synergizes with the signal delivered through the BCR to drive B cell activation and differentiation in the absence of T cell help. Upon activation, B cells migrate to the outer T zone, and then move to the red pulp as they differentiate into plasma cells. Antibody responses to LPS do not require T cells but their presence can influence the response. Although development of germinal centers and memory B cells are unusual in these responses, they can occur if primed T cells are available to provide B cells with help.

5.7.2 Cellular and Molecular Requirements for Efficient TI-2 Responses

T-independent antigens consist of pure polysaccharides, such as those present in the capsules of *Haemophilus influenzae* type b, *Neisseria meningitidis* and *Streptococcus pneumoniae*. The defining characteristics of TI-2 antigens are their high molecular weight, repetitive epitopes and resistance to degradation *in vivo*. They cause extensive cross-linking of B cell receptors (BCR), but are poorly internalized by B cells [216]. The potency and persistence of the signal through the BCR after ligation by TI-2 antigens probably obviates the requirement for T cell help, which in any case is unavailable because B cells do not process and present epitopes from polysaccharides to T cells.

B cell activation and antibody production occurs earlier than in TD B cell responses as the T cell priming stage is not necessary [217]. While this rapid antibody response may be crucial for host defense, TI-2 antigens fail to stimulate high affinity memory B cells so repeated exposure to TI-2 antigens does not evoke an anamnestic response. This is because germinal centers, which are the sites of memory B cell formation, are unusual in responses to TI-2 antigens, and when they do form they appear to involute before memory cells are generated [199]. Nevertheless, TI-2 antigens stimulate a long-lived antibody response, probably due to ongoing B cell activation by persistent polysaccharide antigen [217].

Early studies using sublethally irradiated rats showed marginal zone reconstitution correlated with the acquisition of responsiveness to polysaccharide antigens [218]. More recent studies in mice lacking marginal zones due to disruption of the Pyk-2 gene have confirmed the requirement of this B cell subset to mount TI-2 responses [144]. The marginal zone B cell population is mainly present in the spleen, and constitutes approximately one third of all human splenic B cells.

B-1 cells generate natural IgM antibody, which is known to bind capsular polysaccharides efficiently.

These antibodies may contribute to responses against encapsulated pathogens, however, the finding that mice deficient in B-1 cells still mount normal TI-2 responses indicate that this B cell subset is not essential in protection against encapsulated bacteria [134].

Since CBA/N mice exhibit specific unresponsiveness to TI-2 antigens, they provide a crucial model for elucidating the molecular requirements of these responses. In Xid mice, there is a reduction in the size of the recirculating follicular population [219] and the recirculating cells have a curtailed life-span [220]. Examination of the spleen reveals preservation of marginal zones. Xid mice have absent B-1 cells, although this is likely to be a consequence of the signaling defect, rather than an explanation of TI-2 unresponsiveness (Table I). After stimulation with polysaccharide antigen, Xid B cells migrate to the outer T zones and complete cell cycle but fail to differentiate into plasma cells. Failure to undergo terminal differentiation is associated with a selective defect in Blimp-1 upregulation [143].

The cause of the CBA/N defect has been identified as a point mutation in the gene encoding Bruton's tyrosine kinase (Btk) [221]. Initial phosphorylation of Btk upon BCR engagement is dependent on Syk. The protein BLNK functions as an adaptor of Syk in a signaling complex involving Btk, vav and Grb-2 [222]. The phenotype of BLNK deficient mice is strikingly similar to Xid [223]. Several isoforms of protein kinase C (PKC) and phosphoinositol-3 (PI 3) kinase are co-activators of Btk. The PI 3 kinase product PIP-3 interacts with Btk via its pleckstrin homology and participates in its localization to the cell membrane. The phenotype of PKCβ- [224] and PI 3-kinase-deficient mice [225] is also similar to Xid. Btk participates in several signaling pathways downstream from the B cell receptor (BCR), which are critical for survival and differentiation of activated B cells in response to polysaccharide antigens. Btk directly promotes PLC-γ activation. This results in inositol triphosphate release and initiation of intracellular calcium fluxes in response to BCR crosslinking. Mice deficient in PLC-γ also present with a B cell immunodeficiency similar to Xid [226].

TI-2 responses are abrogated in TACI -deficient mice. TACI is a receptor for BAFF, which is highly expressed on T cells, dendritic cells and macrophages. This finding provides evidence for non-T cell derived accessory signals during T-independent B cell-activation. Since macrophage depletion does not compromise TI-2 responses [227, 228], it seems likely that dendritic cells may provide this accessory signal.

6. MOLECULAR AND ARCHITECTURAL CORRELATES OF B CELL PATHOLOGY

6.1 Primary B Cell Immunodeficiency Diseases

Most primary immunodeficiency diseases (PID) are rare but consideration of these conditions provides important information about the normal function of the immune system. For PID where the molecular defects have been characterized, it becomes possible to make specific correlations between molecular function and lymphoid organization (Table IV).

6.1.1 X-linked Agammaglobulinemia

A form of congenital antibody deficiency presenting in young boys was identified half a century ago and named eponymously as Bruton's agammaglobulinemia, and is now known as X-

Table IV. B cell immunodeficiency and lymphoid architecture.

Diagnosis	Defect		
	Functional	Molecular	Architectural
X-Linked Agammaglobulinaemia	Agammaglobulinemia	Btk BLNK	Absent follicles Absent plasma cells
Autosomal Recessive Hyper IgM syndrome	Low IgG and IgA Normal or high IgM / IgD	Activation-induced cytidine deaminase (AID)	Large germinal centers
X-linked Hyper IgM syndrome	Low IgG and IgA Normal or high IgM / IgD	CD154 (CD40L)	Absent germinal centers
Common Variable Immunodeficiency	Hypogammaglobulinemia	?	Follicular hyperplasia 'Burnt out' germinal centers Reduced plasma cells
Normal infancy	Absent polysaccharide response	?	Absent marginal zone

linked agammaglobulinemia (XLA) [229]. This condition is characterized by a near total absence of recirculating mature B cells and very low or undetectable levels of serum immunoglobulin. In XLA, secondary lymphoid organs are virtually devoid of B cells. Follicles are very scarce and poorly formed, and both germinal centers and plasma cells are absent. In untreated XLA, the majority of infections involve the upper and lower respiratory tracts, resulting in chronic bronchitis and pneumonia, which leads to bronchiectasis and cor pulmonale. Infections with encapsulated organisms, which normally evoke TI-2 responses (*Section 5.8*), predominate. Enteroviruses also pose a significant risk, and echovirus infection may lead to chronic meningoencephalitis. This may also reflect the absence of TI-2 responses since there is evidence that enteroviruses can activate B cells independently of T cells help by virtue of the regular array of antigens on the viral capsid [230]. The prognosis of XLA has improved dramatically with the introduction of immunoglobulin replacement therapy.

In 1993, point mutations in a novel tyrosine kinase (named Bruton's tyrosine kinase, Btk) [221, 231] were identified in patients with XLA. As noted above, this is the same molecule that is defective in X-linked immunodeficiency (Xid) (CBA/N) mice, which exhibit a specific defect in TI-2 responses [231]. While XLA patients have severe B cell deficiency, the few B cells that are present exhibit a selective defect in TI-2 activation [232]. CBA/N mice have a less severe form of B cell immunodeficiency than humans with XLA. In XLA, there is near total arrest of B cell development in the bone marrow at the pre-B cell stage of ontogeny, and very few mature B cells are found in the periphery. In Xid mice there is a more subtle arrest at the pre-B cell stage, but the most obvious defect is in the transition from immature to mature cells [233, 234].

Female carriers of *Btk* mutations do not manifest B cell deficiency, even though approximately 50% of their B cell precursors express the mutant gene. This is because B cell precursors that express the normal allele are at a selective advantage and generate the B cell repertoire. However, not all humans with congenital agammaglobulinemia are male, indicating that defects in other genes can lead to a similar phenotype. Recent studies have identified novel mutations in molecules involved in the Btk pathways of B cell activation in humans and mice. In humans, inactivation of the linker molecule BLNK has been shown to cause the XLA phenotype [235], and mouse studies have shown that selective TI-2 unresponsiveness also occurs after deletion of phosphoinositol-3 kinase, phospholipase C-γ or protein kinase C-β genes [224, 226]. It is possible that investigation of humans with selective TI-2 unresponsiveness may reveal similar B cell signaling defects.

6.1.2 Hyper IgM Syndrome

Hyper IgM syndrome (HIMS) is a cause of combined immunodeficiency, characterized by IgG and IgA deficiency but normal or increased levels of IgM. Patients with HIMS present with recurrent infections in early childhood, although the spectrum is somewhat different from that observed in individuals with XLA. While recurrent pyogenic infections may dominate the clinical presentation, HIMS patients may also suffer a spectrum of infections that is more typical of T cell deficiency, including *pneumocystis carinii* pneumonia and intestinal cryptosporidiosis. Chronic biliary tract infections with cryptosporidia and microsporidia also pose a significant problem; secondary biliary cirrhosis due to chronic biliary tract infection and obstruction can complicate HIMS.

The presence of serum IgM (and IgD) but not other Ig isotypes reflects a severe defect in isotype switching to IgG, IgA, and IgE. A defective CD40L (CD154) gene on the X chromosome is usually the cause of this phenotype [154]. Consequently, T cell-dependent B cell responses are compromised. In contrast with XLA (and Xid), TI-2 responses are preserved. The majority of serum IgG3 is derived from TI-2 responses and in HIMS IgG3 is usually detectable.

Histological analysis of secondary lymphoid organs from patients with HIMS reveals absent germinal centers (Table IV). This observation was critical in identifying the importance of CD40 ligation for germinal center formation. Subsequent studies of $CD40L^{-/-}$ and $CD40^{-/-}$ mice have confirmed that this ligand interaction is also essential for TD germinal center formation in mice. The presence of isotype switching in response to TI-2 antigens in HIMS confirms earlier evidence that isotype switching is not confined to germinal centers but also occurs in extrafollicular plasma cell responses. Under exceptional circumstances, TI-2 antigens can induce germinal center formation [199]. However, this appears to depend on the presence of a high precursor frequency of antigen-specific B cells and while such a high frequency may rarely arise under normal circumstances, these experiments have revealed that TI-2 germinal centers can be induced in the absence of CD40 ligation.

Although HIMS is a defect of germinal center formation and isotype switching, the clinical presentation is often characterized by infections indicative of T cell deficiency. There are several possible non-mutually exclusive explanations for this. First, HIMS patients remain competent to make TI-2 responses. Second, dendritic cells are also known to express CD40L, and its absence from these cells may result in impairment of cytotoxic T cell activation. Third, CD40 ligation may play an important role in activating endothelium and

epithelium to prevent infections at the surface of gastrointestinal and respiratory tracts. Finally, CD40L on T cells may transmit a signal for normal T cell activation.

As with XLA, not all HIMS patients are male. Recently, defects involving *activation-induced cytidine deaminase (AID)* have been identified in patients with HIMS with an autosomal recessive pattern of inheritance [236]. As described in *Section 5.5.2*, AID is expressed specifically in germinal center B cells and is necessary for isotype switching. Its absence leads to production of germline transcripts but isotype recombination is not completed. AID is also necessary for somatic mutation in response to immunization with protein antigens [184]. By contrast with HIMS due to CD40L-deficiency, AID-deficiency is associated with large germinal centers. Differentiation of germinal center B cells is dissociated from somatic hypermutation resulting in futile germinal center formation in the autosomal recessive form of HIMS. Interestingly, patients with autosomal recessive HIMS do not experience opportunistic infections typical of T cell deficiency, which would seem to indicate that in X-linked HIMS, these infections reflect the non-B cell effects of CD40L-deficiency.

6.1.3 Recurrent Infection with Polysaccharide Encapsulated Organisms (Defects of TI-2 Responses)

The most important cause of failure of B cells to respond to polysaccharides is young age. Although humans are capable of generating antibodies to protein antigens from birth, the ability to make antibodies to polysaccharide antigens does not start to develop until after two years of age and does not reach adult levels until approximately five years of age. Polysaccharide unresponsiveness during infancy coincides with the peak incidence of invasive infections with encapsulated organisms [237] and correlates with absence of marginal zone B cells [238].

Several hypotheses have been put forward, including the immaturity of B cells, lack of diversity of the neonatal B cell repertoire, absence of B cells with a marginal zone phenotype, lack of a stromal component in the neonatal spleen, or deletion of polysaccharide-specific B cells to prevent autoimmunity from recognition of cross-reactive neuronal polysaccharide epitopes. Specific failure to produce antibodies to polysaccharides may occur in several contexts. Inability to produce antibodies against polysaccharides may occur in the context of other defined immunodeficiency diseases, including XLA, Wiskott-Aldrich syndrome and ataxia telangiectasia [239]. Acquired susceptibility to encapsulated organisms accompanies hyposplenism. The absence of the spleen increases both the risk of infection with encapsulated organisms and the risk of mortality from invasive disease [240]. This increased risk appears to reflect the importance of the splenic marginal zone for host defense against encapsulated pathogens.

Isolated failure to produce anti-polysaccharide antibodies may also occur as a discrete but poorly understood immune defect. One intriguing subset of patients has excessive $CD5^+$ B cells, suggesting preferential selection into the B-1 compartment [241]. This is the opposite of the situation observed in Xid mice, and the molecular explanation remains unclear.

Recurrent encapsulated bacterial infection in adults may signal the development of common variable immunodeficiency (CVID). This is the most common form of primary antibody deficiency and is a heterogeneous group of disorders [242]. CVID can present at almost any age, most commonly in early adulthood. The spectrum of infections is similar to that in XLA, although some patients also exhibit T cell defects. By contrast with XLA, peripheral B cell numbers may be normal in CVID but B cells lose the capacity to differentiate into plasma cells and secrete Ig. Histological examination of the secondary lymphoid organs reveals follicular hyperplasia but germinal centers are small or burnt-out and plasma cells are reduced or absent, reflecting a defect in differentiation in response to antigen. Sometimes, CVID presents with a sarcoid-like illness, and there is evidence suggesting that this is associated with high levels of TNF due to a polymorphism of the TNF gene (TNF 488A) [243]. In this subset, follicles can be replaced with non-caseating granulomata.

The presence of normal numbers of circulating B cells in the absence of differentiation into antibody forming cells, and the recent demonstration that CVID B cells exhibit a defect in CD86 upregulation after BCR ligation raises the possibility that B cells have become anergic [244]. Alternatively, the transformation of follicles into granulomata would be consistent with an autoimmune response towards follicle components that precludes terminal B cell differentiation.

6.2 Inflammation as an Excessive Response to Antigen

6.2.1 Non-neoplastic B Cell Expansion in Reactive Lymph Nodes

In conditions associated with reactive lymph node hyperplasia, an increase in the size and number of lymphoid follicles indicates that B cell proliferation is taking place. In addition, antigen-driven B cell responses generate secondary follicles with germinal center reactions, and plasma cell expansion. From the principles outlined above, possible explanations for an increase in the size of follicles would include increased selection due to a

Table V. Conditions associated with B cell proliferation.

I. Follicular hyperplasia and germinal center formation
Infections
Bacterial
Viral
EBV
HIV
Syphilis
Autoimmune disease
Rheumatoid arthritis
Hashimotos thyroiditis
Sjogren's syndrome
Systemic lupus erythemotosus
Addison's disease
Other
Chronic benign lymphadenopathy of childhood
Progressive transformation of germinal centers
II. Conditions associated with polyclonal plasmacytosis
Rheumatoid arthritis
Sjögren's syndrome
Castleman's disease
Angioimmunoblastic lymphadenopathy with dysproteinemia (AILD)
Benign polyclonal hypergammaglobulinemia
NF-ATc1/c2$^{-/-}$
IL-6R transposon/ IL-6 transgenic
Fas mutants (*lpr*)
Fas Ligand mutants (*gld*)
Lyn$^{-/-}$
FcγRIIB$^{-/-}$

reduction in the BCR signaling threshold (eg Aiolos$^{-/-}$ mice), or excessive intrafollicular chemokine secretion.

In broad terms, follicular hyperplasia is observed with infections, autoimmune disease, and several diseases of unknown etiology (Table V). For example, streptococcal pharyngitis causes marked follicular hyperplasia in draining cervical lymph nodes. Viremia can cause similar changes generalized to all lymph nodes. This is the case with both EBV and HIV infection [245]. Lymphoid follicles are thought to be an important reservoir of HIV due to the presence of FDCs. CXCR4 is a co-receptor for HIV and is expressed by B cells at all stages of ontogeny, therefore it is plausible that HIV itself acts as a chemoattractant for B cells within secondary lymphoid tissue.

Rheumatoid arthritis (RA) is associated with marked follicular hyperplasia as well as substantial plasma cell expansion in medullary cords, which may extend into the paracortical areas of lymph nodes. Similar changes are observed in Sjögren's syndrome, Hashimoto's thyroiditis, and autoimmune adrenalitis (Addison's disease). These findings suggest that B cell activation is important in the pathogenesis of these conditions and correlates with the prominent autoantibody response.

6.2.2 Splenic Reactive B Cell Diseases

In healthy adult spleen, there is scarcely any evidence of antigen-driven B cell responses because germinal centers and plasma cells are rare. This is because intact antigen normally enters the spleen from the blood so splenic follicular hyperplasia is only observed in conditions associated with systemic antigenemia, such as chronic septicemia, subacute bacterial endocarditis, chronic meningococcemia, and some systemic viral infections. Systemic autoimmune disease such as RA can also lead to splenic follicular hyperplasia and plasma cell expansion.

6.2.3 Non-neoplastic Plasma Cell Proliferation

Disorders associated with polyclonal plasma cell expansion and hypergammaglobulinemia are listed in Table V. Since differentiation to plasma cells represents one of the terminal events of B cell activation, there is some overlap between conditions associated with increased plasma cells and those associated with follicular hyperplasia. This may be a reflection of chronic antigen stimulation, particularly if it occurs in the presence of a lowered BCR signaling threshold. Predominance of plasmacytosis would be expected to result from aberrant expression of extrinsic signals known to drive plasma cell differentiation (eg IL-6, CD70), over-expression of the B cell-intrinsic molecules responsible for programming plasma cell differentiation (Blimp-1, BSAP), failure to activate germinal center differentiation after stimulation with antigen (Bcl-6 defects), or prolonged plasma cell survival (CD11c^{hi} junction zone dendritic cell expansion).

Mice transgenic for IL-6 develop massive plasmacytosis involving lymphoid and non-lymphoid parenchyma [246]. A similar phenotype is observed with Castleman's disease. A subset of these patients is infected with human herpes virus 8, which secretes viral IL-6 [247, 248]. A candidate source of IL-6 production in secondary lymphoid organs is the CD11c^{hi} subset of dendritic cells, which are found in the extrafollicular sites of plasma cell differentiation. Experimental manipulation of the size of this population has

revealed a close correlation with the size of the extrafollicular plasma cell response [204, 205].

Mice bearing mutations of either Fas or Fas ligand (FasL) develop hypergammaglobulinemia, with autoantibody production [249] although B cell activation depends on the presence of Fas/FasL-deficient T cells. A similar phenotype has been observed in children with autoimmune lymphoproliferative syndrome (ALPS, Canale-Smith syndrome) [250].

Evidence that B cell intrinsic signaling defects can cause plasmacytosis has been recently provided by NFATc1/c2$^{-/-}$ mice [251]. Analogous defects in human disease have not yet been described.

6.3 Ectopic Reactive Lymphoid Hyperplasia

In diseases associated with chronic inflammation, lymphocytes can become organized into lymphoid aggregates, segregate into T and B cell zones, and even form highly sophisticated structures such as germinal centers within non-lymphoid parenchyma (Table VI). Some evidence suggests that lymphoid neo-organogenesis reflects the intensity and the nature of the inflammatory stimulus [252]. In these conditions, the molecular basis of lymphoid organogenesis might be expected to explain the development of ectopic lymphoid tissue. In other words, inflammation may represent a recapitulation of the ontogeny of secondary lymphoid organs [252, 253].

a. Rheumatoid Arthritis

Ectopic germinal centers in sites of inflammation were first recognized in rheumatoid synovium [254]. Ectopic germinal center formation has also been reported in murine models of rheumatoid arthritis [255]. Immunophenotyping of lymphocytes within rheumatoid infiltrates has demonstrated that the synovial microenvironment can support B cell proliferation and differentiation. Molecular characterization of the Ig variable regions from ectopic germinal center B cells suggests that these ectopic structures can also support somatic mutation. Furthermore, IgV region analysis is consistent with antigen-driven mutation and selection. Plasma cells are also found throughout the synovium as the inflammation progresses. IgV sequence analysis has shown that the plasma cells are clonally related to B cells in the same synovial infiltrate indicating that B cell differentiation proceeds *in situ* [256].

b. Sjögren's Syndrome

Ectopic T and B cells and germinal centers are found in salivary, lacrimal and other exocrine glands from patients with Sjögren's syndrome, and these reactions are responsible for autoantibody production [257]. Somatic mutations have also been identified in germinal center B cells and plasma cells microdissected from salivary gland biopsies. As with rheumatoid synovium, the ratio of replacement to silent mutations within IgV regions indicate that centrocyte selection is taking place [258].

c. Myasthenia Gravis

Myasthenia gravis is an antibody-mediated disease in which high affinity IgG antibodies are targeted to the acetylcholine receptor (AChR), causing inflammation at the motor end-plate and blocking neuromuscular transmission. Germinal centers within ectopic thymic B cell follicles have long been associated with myasthenia gravis [259]. Although the thymus is a lymphoid organ, these germinal centers are ectopic because the thymus is a primary lymphoid organ and is not usually the site

Table VI. Autoimmune diseases associated with ectopic follicular hyperplasia and germinal center formation.

Disease	Location	Reference
Rheumatoid arthritis	Synovium	[254]
Sjogrens syndrome	Salivary, lacrimal, and other exocrine glands	[257]
Hashimoto's thyroiditis	Thyroid	[279]
Chronic hepatitis	Liver	[280]
Uveoretinitis	Choroid	[281]
Cryptogenic fibrosing		
Alveolitis	Lung	[282]
Myasthenia gravis	Thymus	[283]
Addison's disease	Thymus	
Graves' disease	Thymus	
SLE	Thymus	

of immune responses to antigen. B cells activated within thymic germinal centers are responsible for production of pathogenic antibodies against the AChR [260]. Thymic germinal centers appear to be similar to germinal centers in secondary lymphoid organs except for the failure of high affinity self-reactive mutants to undergo negative selection [261].

Ectopic thymic follicles containing germinal centers are not exclusive to myasthenia gravis. They have also been observed in other autoimmune diseases, including Addison's disease, Graves' disease, rheumatoid arthritis and SLE. However, the antibodies derived from thymic germinal centers in these diseases have not been well characterized.

d. Other Diseases Associated with Ectopic Lymphoid Tissue

Follicular hyperplasia with germinal center formation has also been observed in the thyroid in Hashimoto's thyroiditis, the choroid plexus of the eye in uveitis, and the lung in cryptogenic fibrosing alveolitis. These lesions have not been analyzed by molecular techniques. Ectopic germinal centers have also observed within the inflammatory infiltrate that forms during chronic *H. pylori* gastritis [262] (Table VI).

6.3.2 Ectopic Lymphoid Tissue Development and Disease Pathogenesis

Interactions between hematopoietic and mesenchymal (stromal) cells appear to be responsible for establishing primordial FDC structures that secrete chemokines, which in turn attract lymphocytes (*Sections 2 and 3*). Since lymphocytes themselves contribute to the organization of the mature structure, it is plausible that the same process takes place in sites of chronic inflammation.

Ectopic lymphoid tissue may help explain disease pathogenesis in several ways. First, the positive feedback loop that guides lymphoid organogenesis means inflammation may be self-perpetuating. Second, while ectopic lymphoid organs bear many of the hallmarks of normal lymphoid tissue, their precise immunoregulation may be different. This may result in failure to censor autoreactive B cells with the same efficiency as occurs in normal lymphoid organs resulting in production of autoantibodies. There is evidence that autoantibodies are generated in the germinal centers of RA and myasthenia gravis, and in thymic germinal centers. B cells fail to downregulate Bcl-2 normally, which may contribute to defective negative selection of centrocytes [261]. Finally, the presence of ectopic lymphoid tissue may also contribute to neoplastic transformation. There is an increased incidence of lymphoma in many of the diseases associated with ectopic lymphoid tissue formation. For example, marginal zone B cell lymphoma occurs with increased frequency in the context of Sjögren's syndrome, Hashimoto's thyroiditis and *H. pylori* infection [263]. Since somatic mutation is active in these sites, the association is biologically plausible. Furthermore, it may be that in ectopic lymphoid tissue in which immune responses are driven by chronic self-antigen stimulation where regulatory mechanisms are less developed, the capacity to censor neoplastic lymphocytes is compromised.

6.3.3 Mechanisms of Ectopic Lymphoid Tissue Development

In chronic inflammatory diseases where complex infiltrates of inflammatory cells become organized into structures that resemble secondary lymphoid organs, the molecular interactions guiding neo-organogenesis are likely to be similar to those that act during development of normal lymphoid organs.

Based on knowledge of secondary lymphoid organ development (*section 2*; Figs. (**4**) and (**5**)), lymphoid neo-organogenesis at sites of chronic inflammation would be expected to be a two-phase process. First, interactions between hematopoietic and mesenchymal cells establish rudimentary structures then lymphoid cells become organized into follicles competent to support germinal centers guided by on-going hematopoietic cell-mesenchymal cell interactions, and expression of chemokines. It would be predicted therefore, that the crucial regulators of lymphoid neo-organogenesis (involving B cells) would be ligation of IL-7Rα or RANK (on lymphoid progenitors), upregulation of adhesion molecules MAdCAM-1, VCAM-1, or ICAM-1 on inflamed mesenchyme (or high endothelial venules), ligation of LTβR in inflamed mesenchyme, and upregulation of CXCL13. According to this model, lymphoid progenitors would locate ectopically in inflamed non-lymphoid tissues where VCAM-1 and ICAM-1 are expressed, and ligation of LTβR in mesenchymal cells by common lymphoid progenitors would lead to CXCL13 production within the tissue. It is therefore possible that antigenic insults may cause transient inflammation and *de novo* CXCL13 secretion from thyroid, synovial or pancreatic stromal cells, which in turn may lead to neo-follicularization in these organs and B cell activation *in situ* [27].

There are two lines of evidence that support this scenario. First, genes encoding key regulatory molecules have been engineered to be expressed ectopically. Second, expression of key molecules have been found within established ectopic lymphoid tissue.

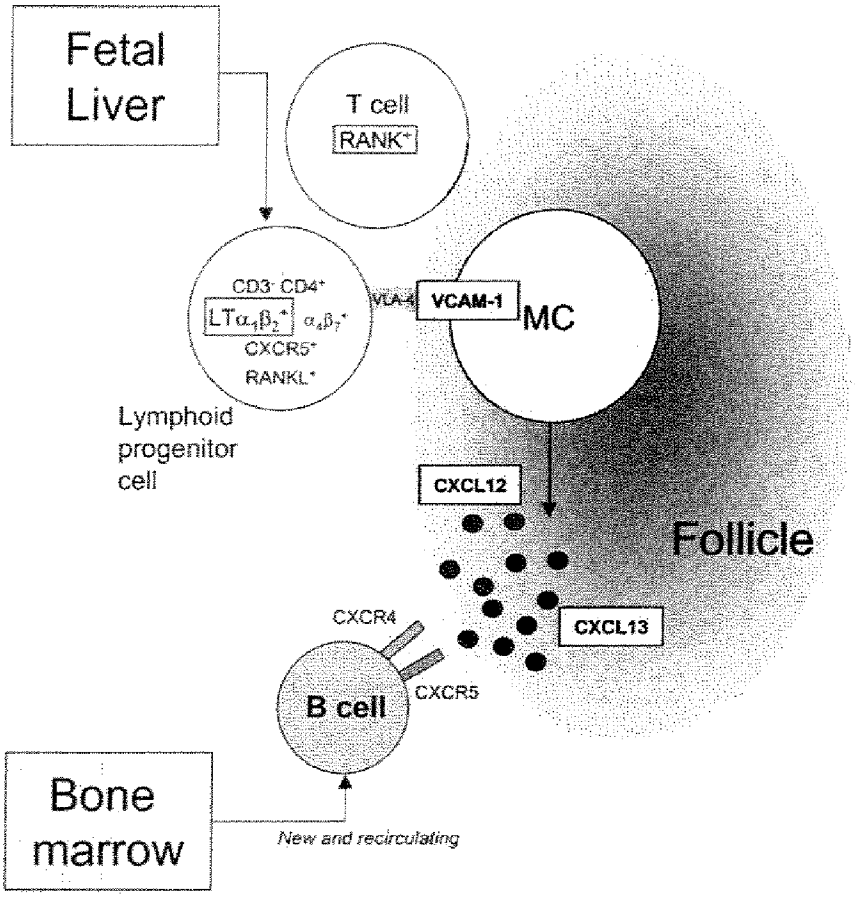

Figure (8). Ectopic lymphogenesis.

Formation of ectopic lymphoid follicles may follow the same scheme lymphoid organogenesis (*see text and legend to Fig. 4*). Molecules identified so far within ectopic lymphoid tissue are shown in boxes.

a. Ectopic Expression of Molecules Involved in Lymphoid Development

Follicles appear *de novo* in the pancreas when a transgene for CXCL13 is expressed locally, and this effect is $LT\alpha_1\beta_2$-dependent [28]. Indeed, there appears to be a positive feedback loop driving lymphoid development, since CXCL13 recruits B cells and induces $LT\alpha_1\beta_2$ expression on B cells, and $LT\alpha_1\beta_2$ promotes CXCL13 expression [40], Fig (**8**).

Ectopic expression of CCL21 has also been shown to drive extra-nodal lymphoid neo-genesis [264]. CCL21 binds to CCR7, and although this interaction does not appear to be essential for normal lymphoid development, it appears to drive ectopic lymphoid formation. This may be significant, because if this ligand pair is not essential for normal lymphoid development, it may be an attractive therapeutic target.

Lympoid neo-organogenesis may not always be maladaptive. Therapy with antibody-$LT\alpha$ fusion protein in the context of melanoma has been shown to induce an adaptive immune response and thus tumor regression via stimulation of lymphoid neo-organogenesis within the malignant tissue [265]. High endothelial venules expressing PNAd and an

infiltrate of L-selectin$^+$ T cells were found in the lymphoid infiltrate.

TNF and LTα are not only crucial during normal lymphoid development but are also pro-inflammatory cytokines liberated by cells of the innate immune system during acute inflammation. These cytokines have many different actions, including amplification of the inflammatory response and stimulation of systemic features of local inflammation, including the acute phase response and fever. In RA, TNF appears to play a central role in disease pathogenesis by virtue of its capacity to activate cells within the inflammatory infiltrate [266]. Delineation of the role of TNF in lymphoid neo-organogenesis in RA remains to be determined. However, experimental arthritis occurs in mice when TNF is expressed ectopically, which also leads to ectopic lymphoid aggregates within these inflamed joints [267]. TNF has been shown to induce FDC differentiation from stromal precursors leading to increased CXCL13 synthesis [40].

b. Evidence from Analysis of Pathological Inflammatory Tissue

In RA synovium, fibroblasts express VCAM-1 and secrete CXCL12 [268]. Isolated synovial fibroblasts are capable of providing trophic support for B cells *in vitro*, and this support is abrogated when CXCR4, which binds CXCL12, is blocked with antibodies or pertussis toxin [268]. These findings are consistent with the hypothesis that the rheumatoid synovium provides a signal for lymphoid progenitor recruitment, as well as a milieu that provides trophic support for B cells.

RANK (OPGL) is expressed by activated T cells within the rheumatoid synovial infiltrate. This is a significant finding because RANK activates osteoclasts and its expression may help to explain the resorption of periarticular bone in RA [38]. In addition, based on evidence discussed in *Section 2*, expression of RANK may also contribute to lympoid progenitor recruitment and formation of lymphoid follicles within the synovium. Finally, examination of gastric tissue obtained from patients with chronic *H. pylori* infection has identified CXCL13 expression within ectopic lymphoid tissue in the gastric mucosa [269].

CONCLUDING REMARKS

Fundamental discoveries about lymphoid organogenesis and establishment of the B cell repertoire are revealing novel mechanisms of pathology. Molecular descriptions of organogenesis also provide insights into well-established histopathological findings in immune mediated disease. Nevertheless, the number of immune-mediated diseases that can be explained in precise molecular detail remains small. Further progress will depend on applying other principles of fundamental biology to well-characterized human disease.

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LIST OF ABBREVIATIONS

BCR = B cell receptor

FDC = Follicular dendritic cell

HIMS = Hyper IgM syndrome

IDC = Interdigitating dendritic cell

LPS = Lipopolysaccharide

LT = Lymphotoxin

MHC = Major histocompatibility complex

MZ = Marginal zone

RA = Rheumatoid arthritis

SLE = Systemic lupus erythematosus

TCR = T cell receptor

TD = T cell dependent

TI = T cell independent

TNF = Tumor necrosis factor

XLA = X-linked agammaglobulinemia

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